1. From the Editors

In this summer issue we thought we would visit glutathione and the gamma-glutamyl cycle, a well-studied topic yet one that keeps throwing up some controversy. Since Meister and his colleagues proposed the gamma-glutamyl cycle to support amino acid transport over 50 years ago there has been many debates about the function and importance of the gamma-glutamyl cycle, whether it is considered a mechanism for amino acid transport or a facilitator of extracellular signals, gamma-glutamyl amino acids. In the 70s, two discoveries gathered interest: (1) glutathione-S-transferase (GST) was shown to utilize glutathione for conjugation of drugs and carcinogens to facilitate their elimination and (2) studies on the importance of redox chemistry in biological systems led to the notion that oxidative stress is the ultimate culprit of diseases, leading to enhanced efforts to comprehend radical chemistry and a shift away from nutrition uptake and storage.

A controversial view is that antioxidant and conjugation reactions associated with glutathione are secondary as these only utilize a small fraction less than micromoles of the overall glutathione pool, thereby not fully accounting for its high abundance within tissues. Glutathione concentrations in tumor cells are 1-10 mM which is 10,000-fold higher than hydrogen peroxide, the most abundant reactive oxygen species, which is reported to be present at low nanomolar concentrations. This stoichiometric disconnect has not been addressed sufficiently. To emphasis this Flohé (Biochim Biophys Acta., 2013) argues chemical reduction of oxidized molecules by glutathione is too slow to even be biologically relevant and that enzyme catalyzed reactions dominate glutathione flux. In contrast mechanisms reviewed by Liu et al (Adv Cancer Res., 2014) do not consider the concentration difference.

The huge efforts in drug development and cultural opinion devoted to elucidating the potential of antioxidants have led to a literature bias that, in the case of glutathione, may not be sustainable. Nonetheless, given that glutathione synthesis is linked to many key metabolic pathways and altered in various tumor types, there is no doubt a better understanding of enzymes and pathways affecting glutathione flux in cancer cells including glutathione metabolism could lead to an improved understanding of how tumors strike back and leads to tumor resistance. Our chosen reviews and highlight articles shed some light on this interesting topic and we hope we with this issue can stimulate some thoughts and ideas that can generate further debate.

Dr. Gary Patti, our early career scientist profiled in this late summer issue, is engaged with using metabolomics to study how metabolites derived from nutrient intake aid to support the high metabolic demand of cancer cells.

Finally, we are pleased to inform that we from this point onwards will bring information from the CICR Steering Committee under the heading “News from

the CICR Steering Committee". No doubt this will be of great interest to the CICR community and beyond, thereby strengthening the dialogues and debates with focus on chemistry in cancer research! Happy reading!

Editorial co-author: Dr Gunnar Boysen

2. News from the CICR Steering Committee

To solidify and expand the impact of the CICR Working Group, several key initiatives were outlined during the CICR Steering Committee meeting at the AACR Annual Meeting in New Orleans. A follow-up teleconference was held in June during which sub-teams were formed around four primary goals: 1) chemical probes, 2) drug discovery in academia, 3) broadening CICR reach, and 4) promoting the development of the next generation of chemistry-oriented cancer researchers. The latter sub-team will be led by Dr. Sean Kerwin and is focused on ways in which the CICR can help maintain a consistent flow of top-notch chemists engaged in cancer drug discovery. Melissa Vasbinder will lead a sub-team on broadening the reach of the CICR – from a diversity and geographic viewpoint. Drs. Vinny Patel and Paul Hergenrother provide industry and academic perspectives on how the CICR can help drive cancer drug discovery in academic labs. Under Dr. David Uehling's leadership, one of the areas of focus for last year's CICR Steering Committee was the appropriate use of chemical probes. Dr. Uehling will continue this mission by leading the chemical probes sub-team during 2016/17. The success of these initiatives is highly dependent on gaining input from diverse perspectives so CICR members are encouraged to participate by contacting any of these sub-team leaders. For contact details, use this link:
http://www.aacr.org/Membership/PAGES/CICR-STEERING-COMMITTEE.ASPX#.V6CLfxKzurw

3. Selected Glutathione Research Highlights

The fairytale of the GSSG/GSH redox potential (review)
http://www.ncbi.nlm.nih.gov/pubmed/23127894
Flohé L. Biochim Biophys Acta. 2013 May;1830(5):3139-42. doi: 10.1016/j.bbagen.2012.10.020.

Emerging regulatory paradigms in glutathione metabolism (review)
http://www.ncbi.nlm.nih.gov/pubmed/24974179
Liu et al., Adv Cancer Res. (2014) 122, 69-101. doi: 10.1016/B978-0-12-420117-0.00002-5

Glutathionists in the battlefield of gamma-glutamyl cycle (review)
http://www.ncbi.nlm.nih.gov/pubmed/27095217

Inoue M. Arch Biochem Biophys. (2016), 595, 61-3. doi: 10.1016/j.abb.2015.11.023.

Paracrine Induction of HIF by Glutamate in Breast Cancer: EglN1 Senses Cysteine

http://www.ncbi.nlm.nih.gov/pubmed/27368101

Briggs e*t al*. investigated the normoxia-derived mechanism of hypoxia inducible factor (HIF) expression in triple negative breast cancer (TNBC) cell lines. Initially, they identified that the expression of HIF1a was upregulated in several TNBC cell lines under normoxic conditions. However, the HIF1a expression was promoted only when freshly plated TNBC cells were exposed to the "TNBC-conditioned media" rather than to fresh media. They then identified that L-glutamate was the factor that was secreted from TNBC cells. The increased level of extracellular L-glutamate subsequently inhibited the function of the xCT cysteine-glutamate antiporter, resulting in the intracellular cysteine depletion. The cysteine reduction inactivated EglN1, which in turn downregulated the degradation of HIF and thus stabilized HIF1a in TNBC cells. Overall, this study reveals the paracrine mechanism that is responsible for the normoxic accumulation of HIF1a in TNBC cells.

Briggs *et al.* Cell (2016) 166, 126-139. doi: 10.1016/j.cell.2016.05.042.

Glutathione biosynthesis is a metabolic vulnerability in PI3K/Akt-driven breast cancer

http://www.ncbi.nlm.nih.gov/pubmed/27088857

Lien *et al.* reports that oncogenic PI3K/Akt pathways stimulate glutathione (GSH) biosynthesis through the transcription factor NRF2. The authors used liquid chromatography based tandem mass spectrometry to study the functional association of PI3K/Akt pathways with metabolism in tumor growth. Along with glycolysis, the authors discovered that MCF10A cells expressing oncogenic AKT2(E17K) mutation increased the intracellular level of GSH. The GSH level by AKT2(E17K) was due to the upregulation of the gene expression involved in GSH biosynthesis by NRF2. Since the inhibition of GSH biosynthesis reduced the proliferation of breast tumors harboring a PI3K/Akt pathway mutation in both 2D- and 3D-culture systems, these results reveal the important of GSH biosynthesis for treating the PI3K pathway mutant breast cancers.

Lien *et al.* Nature Cell Biol. (2016) 18, 572-578. doi: 10.1038/ncb334.

A Synthetic Lethal Interaction between Glutathione Synthesis and Mitochondrial Reactive Oxygen Species Provides a Tumor-Specific Vulnerability Dependent on STAT3

http://www.ncbi.nlm.nih.gov/pubmed/26283727

Garama *et al.* investigated the oncogenic connection between glutathione (GSH) biosynthesis and mitochondrial STAT3-dependent metabolic processes in Ras-transformed cells. A global metabolite analysis using mass spectrometry identified that the most metabolic intermediates of gamma-glutamyl cycle were

upregulated in the absence of STAT3. However, the precursor and the end product of gamma-glutamyl cycle, cysteine and glutathione, were significantly reduced in STAT3-null cells. Pharmacological inhibition of gamma-glutamyl cycle with small molecules resulted in the depletion of glutathione and the accumulation of reactive oxygen species, followed by cell death. Interestingly, these observations are mitochondrial STAT3-dependent in Ras-transformed cells. Taken together, these data reveal a therapeutic potential of mitochondrial STAT3-dependent GSH biosynthesis at least for a subset of human cancers.
Garama *et al.* Molecular and Cellular Biology (2015) 35, 3646-3656. doi: 10.1128/MCB.00541-15.

Glutamine drives glutathione synthesis and contributes to radiation sensitivity of A549 and H460 lung cancer cell lines.
http://www.ncbi.nlm.nih.gov/pubmed/26825773
The first step in glutamine utilization is its conversion to glutamate by glutaminase (GLS). Using H460 and A549 none small cell lung tumor cell line they show a significant correlation between glutamine consumption and glutathione. Culturing in the presence of [$^{13}C_5$]glutamine demonstrated that by 12h >50% of excreted glutathione was derived from glutamine. Inhibition of GLS reduced cell viability and abolished glutathione excretion and radiosensitized the lung tumor cell lines, suggesting an important role of glutamine-derived glutathione for viability resistance. This is the first study showing that significant amounts of extracellular glutathione is directly derived from glutamine.
Sappington et al. Biochim Biophys Acta. (2016), 1860(4):836-43. doi: 10.1016/j.bbagen.2016.01.021.

Other research highlights:

PAQR3 suppresses the proliferation, migration and tumorigenicity of human prostate cancer cells
http://www.ncbi.nlm.nih.gov/pubmed/27275543
A seven-transmembrane protein localized to the Golgi, PAQR3 is a recently discovered tumor suppressor in a number of different types of cancer. Huang et al. have recently shown that overexpression of PAQR3 in mouse xenograft models of prostate cancer suppresses tumor formation, while knockdown promotes growth of the tumor. PAQR3 expression also suppresses markers of the epithelial-to-mesenchymal transition, and PAQR3 overexpression shows reduced migration of prostate cancer cells ex vivo. These data imply that PAQR3 may be a good target for therapeutic intervention in prostate cancers.
Huang et al., Oncotarget (2016) Jun 3. doi: 10.18632/oncotarget.9807

PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer
http://www.pnas.org/content/113/26/7124.abstract
Raina et al., show that a PROteolysis TArgeting Chimera (PROTAC) is active in models of prostate cancer. The molecule, ARV-771, binds to both the van Hippel-

Lindau ubiquitin ligase and BET (bromodomain and extracellular-terminal) proteins, and it degrades BET proteins via the ubiquitin-proteasome system. The molecule is active in cultured prostate cancer cells, and it shows enhanced reduction of tumor volume in a mouse xenograft model of prostate cancer, relative to a BET inhibitor and to other standard-of-care treatments.
Raina et al., Proc. Natl. Acad. Sci. U.S.A. (2016) 113 (26), 7124–7129, doi: 10.1073/pnas.1521738113

Proteogenomics connects somatic mutations to signalling in breast cancer
http://www.ncbi.nlm.nih.gov/pubmed/27251275
A major goal of the NCI Cancer Proteomics Tumor Analysis Consortium (CPTAC) is to integrate genomic and proteomic data on tumor specimens. To this effect, a recent study from this group described an integrated study of somatic mutations and proteomics data. Proteomic and phosphoproteomic studies were performed to analyze >100 breast tumors with genomic annotations. This integrated approach allowed proteomics to provide new molecular insights into genomic observations. For example the 5q deletion commonly observed in basal-like breast cancer was associated with the loss of CETN3 and SKP1 and connected to elevated EGFR expression. SKP1 loss was also associated with increased SRC tyrosine kinase. A group of stromal-enriched proteins were also confirmed by the proteomics data and a novel G-protein-coupled receptor cluster, not identified at the mRNA level, was observed at the phosphoproteomics level. Furthermore, ERBB2 and other amplicon associated highly phosphorylated kinases were identified. This study provides insight into the proteomic effects, from which functional consequences can be inferred, of somatic mutations. The authors also suggest that driver gene candidates can be refined and therapeutic targets can be identified from this data.
Mertins et al. Nature. (2016) 534(7605), 55-62. doi: 10.1038/nature18003.

Targeted proteomics identifies liquid-biopsy signatures for extracapsular prostate cancer
http://www.ncbi.nlm.nih.gov/pubmed/27350604
Despite ongoing discovery efforts, novel clinical cancer biomarkers remain elusive. Many biomarkers are not validated in independent patient cohorts, do not undergo rigorous evaluation, or fail to provide clinical utility. In a recently published paper, Kim et al. describe an approach combining targeted proteomics and computational biology to identify new biomarkers and protein signatures for prostate cancer. The authors targeted 133 differentially expressed proteins from a previous study where quantitative proteomics analysis of prostatic secretions from men with extraprostatic and organ-confined prostate cancer was performed. Using synthetic peptides corresponding to the 133 proteins, targeted proteomics was performed on expressed prostatic secretions in urine in a 74 patient cohort. A panel of 34 candidates was further validated in an independent cohort of 207 patients. Clinical predictive models for prostate cancer diagnosis and prognosis were further developed using machine-learning. This study represents an approach that uses computationally guided proteomics to discover potential

prostate cancer biomarkers.
Kim et al. Nat Commun. (2016) 7, 11906. doi: 10.1038/ncomms11906.

Integrated Proteogenomic Characterization of Human High-Grade Serous Ovarian Cancer.
http://www.ncbi.nlm.nih.gov/pubmed/27372738
A major goal of the NCI Cancer Proteomics Tumor Analysis Consortium (CPTAC) is to integrate genomic and proteomic data on tumor specimens. To this effect, a recent study from this group described a study integrating such data in ovarian cancer. Mass spectrometry was used to perform in-depth proteomic analysis of 174 ovarian tumors that had been previously analyzed by The Cancer Genome Atlas (TCGA), with the majority of the tumors being high-grade serous carcinomas. The data revealed new aspects of ovarian cancer, including for example, the influence of copy-number alterations on the proteome. The study also showed that acetylation of a specific protein, histone H4, was correlated with homologous repair deficiency status. In addition the abundance of proteins and phosphoproteins were linked to pathways associated with survival. These results have potential applications in stratifying patients for therapy, and provide new information about how the genome drives the proteome in high grade serous ovarian cancer.
Zhang et al. Cell. (2016), pii: S0092-8674(16)30673-0. doi: 10.1016/j.cell.2016.05.069.

4. Profile of a Young Scientist

	Employment	
	Jan 2015-present	Associate Professor; Washington University in St. Louis
	Nov 2011- Jan 2015	Assistant Professor; Washington University in St. Louis
	July 2008 - Nov 2011	NIH Postdoctoral Fellow; Department of Molecular Biology and Center for Mass Spectrometry. The Scripps Research Institute, La Jolla, CA. Advisor: Dr. Gary Siuzdak
	Education	

	2003-2008 1998-2002	Ph.D., Chemistry; Washington University in St. Louis Advisor: Dr. Jacob Schaefer B.A.,Chemistry/Philosophy; Saint Louis University, St. Louis, MO. Undergraduate research advisor: Dr. Shelly Minteer

Dr. Gary Patti is currently an associate professor at Washington University in St. Louis in the departments of Chemistry and Medicine. He trained as a graduate student in the laboratory of Dr. Jacob Schaefer, inventor of cross-polarization magic-angle spinning NMR. There he integrated solid-state NMR and LC/MS technologies to examine alterations in bacterial metabolism related to antibiotic resistance. He joined the laboratory of Dr. Gary Siuzdak at the Scripps Research Institute as a postdoctoral fellow. In Siuzdak's laboratory, he helped develop mass spectrometry-based resources that are widely used in the field of metabolomics (such as the XCMS software and the METLIN metabolite database).

By using cutting-edge metabolomic technologies, thousands of signals can be detected in cancer cells and tissues. Interestingly, many of these signals cannot be identified with current approaches. One focus of the Patti laboratory has been to characterize metabolomic signals that correspond to unknown pathways regulating cancer biology by applying new technologies they have developed (such as X^{13}CMS, credentialing, Warpgroup, and metabolite bar coding). Dr. Patti has been recognized with several awards including the Pew Scholars Award, the Camille Dreyfus Teacher-Scholar Award, the Alfred P. Sloan Foundation Award, the Mallinckrodt Scholar Award, and the Academy of Science St. Louis Innovation Award. His work is supported by an R01 from the National Institute of Environmental Health Sciences and an R21 from the National Cancer Institute.

5. Spotlight on World News

AbbVie's Rova-T Phase I trial generates enthusiasm for treating aggressive SCLC
http://www.biopharminsight.com/abbvies-rova-t-phase-i-generates-enthusiasm-sclc-potential-despite-investor-malaise-oncologists

AbbVie's Rova-T (rovalpituzumab tesirine) has generated excitement among experts for its potential in small-cell lung cancer (SCLC) treatment following Phase I results presented at the recent 2016 American Society of Clinical Oncology (ASCO) Annual Meeting. Rova-T, which is an antibody-drug conjugate (ADC), is seen as a promising new biomarker-directed therapy for treating aggressive SCLC, which accounts for 13-15% of all lung cancers and has a five-year survival rate that is less than 5%. Although the Phase I trial was relatively small it was not designed to detect a marked difference in survival, hence the results are seen as encouraging.
Source: Biopharm Insight

UK charity pockets $150 million from Merck cancer immunotherapy drug
http://www.reuters.com/article/us-merck-britain-charity-idUSKCN0ZU2RD
British medical charity MRC Technology realize $150 million by selling part of its royalty interest in Merck & Co's successful cancer drug Keytruda (pembrolizumab) to a private equity fund managed by DRI Capital. Keytruda is one of a promising class of new treatments for melanoma or lung cancer that stimulate the body's immune system to fight cancer by blocking the PD-1 pathway. The antibody-based medicine was "humanized" by scientists at MRC Technology.
Source: Reuters

6. Career Forum

https://cancercareers.org/Pages/default.aspx

http://www.nature.com/naturejobs/science/jobs

http://jobs.rsc.org/

http://chemistryjobs.acs.org/

7. Conferences

2nd Annual Drug Discovery USA Congress
http://www.discoveryusa-congress.com/
October 3-4, 2016. San Diego, CA, USA

Translational Control of Cancer: A New Frontier in Cancer Biology and Therapy
October 27 - 30, 2016 | San Francisco, California

DNA Repair: Tumor Development and Therapeutic Response
November 2 - 5, 2016 | Montreal, Quebec, Canada

NCRI Cancer Conference
http://www.ncri.org.uk/
November 6-9, 2016. Liverpool, UK

EORTC-NCI-AACR Molecular Targets and Cancer Therapeutics
http://www.aacr.org/Meetings/Pages/MeetingDetail.aspx?EventItemID=61#.V6SVwE-FP5o
November 29-December 2, 2016, Munich, Germany

5th International Conference on Medicinal Chemistry & Computer Aided Drug Designing
http://medicinalchemistry.pharmaceuticalconferences.com/call-for-abstracts.php
December 5-7, 2016. Phoenix, AZ, USA

San Antonio Breast Cancer Symposium
December 6 - 10, 2016 | San Antonio, Texas

Precision Medicine Series: Opportunities and Challenges of Exploiting Synthetic Lethality in Cancer
January 4 - 7, 2017 | San Diego, California

AACR Annual Meeting 2017
April 1 - 5, 2017 | Washington, DC

American Peptide Society Meeting
http://aps2017.org
June 17-22, 2017. Whistler, BC, Canada

8. Other