1. From the Editors

   The goal of targeted cancer therapy (personalized or precision medicine) is to identify tumor features amenable to therapeutic intervention. The implementation of personalized cancer treatment modalities occurs at various levels and antibody–drug conjugates (ADCs) offer much promise as a therapeutic modality. Ultrapotent cytotoxics such as the auristatins, maytansines, calicheamicins, and duocarmycins are small molecules that efficiently eradicate tumor cells in preclinical models, however suffer from off-target toxicities and hence poor therapeutic indices. Encouragingly, when such small molecules are tethered to tumor-selective antibodies their off-target effects are significantly reduced, providing a route to clinical progression of these often pico-to-femtomolar potent cytotoxics. Brentuximab vedotin (Adcetris) and trastuzumab emtansine (Kadcyla) are two ADCs that are already benefiting patients. However, the technologies behind the ADC concept need further improvements in order to more broadly offer cancer patients ADC-based personalised medicine. This newsletter sheds light on some of the most recent advances in ADC technology, including antibodies and linker chemistry, and profiles Dr. David Rabuka, a young scientist in the ADC field.

   At the forthcoming AACR Annual Meeting in Philadelphia (18th-22nd of April), CICR has amongst other things organised four educational sessions under the theme “From Chemistry to the Clinic: Pathways for Drug Discovery and Development”. If you are interested in gaining or updating your knowledge on ADC technologies, make sure to attend Session II: “Discovery and Development of Antibody-Drug Conjugates”, Saturday, April 18, 2015, 10:15 am - 12:15 pm. For other chemistry-related sessions to be held at the Annual Meeting, please see the CICR website.

   See you at the CICR Town Hall at the AACR meeting on Monday, April 20 from noon to 1:30 pm!

2. Selected Research Highlights

   Antibody–drug conjugates: an emerging modality for the treatment of cancer (Review 1)

   Biologics: an update and challenge of their pharmacokinetics (Review 2)

   Site-Specific Antibody–Drug Conjugates: The Nexus of Bioorthogonal Chemistry, Protein Engineering, and Drug Development (Review 3)

   Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates Antibody-drug conjugate (ADC) development involves the selection of a drug linker and attachment chemistry to link a drug molecule to an antibody therapeutic. Maleimide conjugation to

reactive thiols is often used for drug-antibody linking. However, ADCs using this linkage chemistry can undergo deconjugation of the maleimide group in plasma leading to increased toxicity in vivo. Alternatively, the maleimide group can undergo hydrolysis preventing deconjugation from occuring and increasing the linker stability. Recent work by Lyon et al. investigated different linker chemistries to induce the maleimide hydrolysis following conjugation. The addition of a basic amino group adjacent to the maleimide effectively induced hydrolysis of the maleimide at neutral pH preventing future nonspecific drug deconjugation. Dosing with this modified drug linker in vivo showed increased stability characteristics while maintaining similar cleavage assay kinetics at every conjugation site.

Chemically Synthesized Molecules with the Targeting and Effector Functions of Antibodies McEnaney et al. describe the synthesis and assessment of a new class of compounds called synthetic antibody mimics targeting prostate cancer cells. The synthetic antibody mimics have a very small size (~7000 Da) relative to the molecular weight of antibodies. They are composed of two moieties; first, a glutamate-urea motif that binds to prostate-specific membrane antigen (PSMA), which is highly overexpressed on membranes of prostate cancer cells, and second, a cyclic peptide that binds to Fc gamma receptor type I (FcγRI), which is found on the surface of immune cells. When the synthetic antibody mimics bind to the PSMA and also to the FcγRI, phagocytosis was induced in a concentration-dependent manner in prostate cancer cells. However, the compound showed very low levels of phagocytosis in cells that did not express PSMA. Collectively, the authors have demonstrated a novel class of synthetic compounds that function as specific as antibodies to prostate cancer cells.

Exploring the effects of linker composition on site-specifically modified antibody-drug conjugates Non-cleavable linkers are an important component of antibody-drug conjugates, allowing the delivery of a cytotoxic small molecule to a tumor, and avoiding non-specific release of the drug. Non-cleavable linkers also allow the chemical properties of the small molecule to be altered to tune affinity for the transporter or improve potency. Albers et al. describe a panel of site specifically conjugated ADCs, with differing non-cleavable linker portions, containing an aldehyde tag coupled with the hydrazine-iso-Pictet-Spengler (HIPS) ligation. The panel used five different linkers to carry a maytansine payload. These molecules were characterized using in vitro and in vivo assays and only small differences were observed with regards to efficacy. These results suggest that non-cleavable linkers can be tailored with specific chemistries to adjust the potency of ADCs.

Innovative Native MS Methodologies for Antibody Drug Conjugate Characterization: High Resolution Native MS and IM-MS for Average DAR and DAR Distribution Assessment An important attribute of an antibody drug conjugate (ADC) is the drug-to-antibody ratio (DAR) as this determines the

amount of cytotoxic payload present on an antibody therapeutic. Mass spectrometry has often been used as a complementary method to traditional analytical techniques like UV spectrophotometry to determine the drug-to-antibody ratio. Recent work by Debaene et al. demonstrated the use of ion mobility mass spectrometry as an alternative mass spectrometry approach to determine the drug loading in Brentuximab Vedotin, a cysteine interchain-disulfide linked ADC, while also interrogating higher order structure differences in the antibody following drug conjugation. Individual drug loaded forms containing 0, 2, 4, 6, or 8 drug molecules were determined to have different ion mobility drift times and thus different collisional cross sections demonstrating slight confirmational structure changes resulting from the conjugation. The authors developed a semi-quantitative method to calculate the average DAR ratio as well as the overall drug distribution among the various drug loaded forms.

SGN–LIV1A: A Novel Antibody–Drug Conjugate Targeting LIV-1 for the Treatment of Metastatic Breast Cancer Despite numerous treatment options for breast cancer, those with late stage disease continue to have poor survival rates due to a lack of suitable drug targets. Sussman et al. determined that LIV-1, a zinc transporter, is expressed even after hormonal therapy and in triple-negative breast cancer making it a desirable target for those with advanced disease. They developed a novel antibody-drug conjugate (SGN-LIV1A) in which monomethyl auristatin E (MMAE), an antineoplastic agent which disrupts microtubules, is conjugated to a humanized antibody specific for LIV-1. Both in vitro experiments and xenograft mouse models of breast and cervical cancer showed that SGN-LIV1A binds and is taken up by cells expressing LIV-1 and has potent antitumor activity. Authors reported significant regression in tumor growth, particularly in breast cancer models, suggesting that this new ADC could provide an additional treatment option for patients with late stage disease.

Antibody fragment-conjugated polymeric micelles incorporating platinum drugs for targeted therapy of pancreatic cancer Scientists from Japan and China developed antibody fragment-installed polymeric micelles via maleimide-thiol conjugation for selectively delivering platinum drug (DACHPt) to pancreatic tumors. Immunomicelles are synthesized by conjugating anti-tissue factor (TF) antibody fragment (Fab') to maleimide functionalized DACHPt-loaded micelles (~31 nm). Their results show that immunomicelles are readily taken up by TF-overexpressing cancer cells and subsequently deliver DACHPt intra-cellularly. Based on the advantage of using Fab' fragments of antibodies, which has relatively small size, and by using the one-to-one conjugation strategy of Fab' fragments to polymeric micellar surface by maleimide-thiol coupling, researchers report a potentially generic way of targeted delivery of therapeutic agents while enhancing their therapeutic efficacy.

Characterization of Drug-Product-Related Impurities and Variants of a Therapeutic Monoclonal Antibody by Higher Energy C-Trap Dissociation Mass Spectrometry Drug-product-related impurities and variants of therapeutic

antibodies are generated from product degradation, post-translational modifications (PTMs), and/or chemical modifications. Food and Drug Administration (FDA) requires detailed characterization of drug-product-related impurities and variants of antibodies because of the impurities' potential immunogenicity in the clinic. A collaborative research team led by Dr. Liu at Merck Research Laboratories reported the profiling and characterization of drug product-related impurities of an anti-*Clostridium difficile* IgG1 mAb drug substance using two-dimension chromatography coupled with higher energy C-Trap dissociation (HCD) Mass Spectrometry. The accurate mass measurements and the HCD middle-down MS/MS experiments identified that major impurities and variants of the anti-*C. difficile* mAb were degradation species of the heavy chains at residue Asn101 as well as at the hinge region amino acids. Additional impurities were identified as light chain C-terminal truncation at Gly93 and oxidized heavy chains at Met40, Met93, and Met430. The platform could be particularly useful for those low-level impurities and variants that are not suitable for further fractionation and characterization by bottom-up MS.

Combination of antibody targeting and PTD-mediated intracellular toxin delivery for colorectal cancer therapy The bottlenecks of current chemotherapy for treating colorectal cancer are linked to the ineffectiveness of small molecule drugs and the dose-limiting toxicity of these compounds on normal tissues. Gelonin is a 30 kDa N-glycosidase isolated from the seeds of the Himalayan plant Gelonium multiflorum (false lime) with a very potent ribosome inactivating property. However, its clinical translation remains a challenge due to its poor membrane permeability. To overcome this limitation, Shin et al. developed a conjugate of a non-internalizing anti-carcinoembryonic antigen (anti-CEA) T84.66 mAb and the sulfated anionic glycosaminoglycan heparin paired with the conjugate of the cationic TAT protein transduction domain (PTD) and the protein gelonin. It was found that heparin-anti-CEA mAb conjugate binds with high specificity to CEA-overexpressing LS174T colorectal adenocarcinoma and negligible biding to low CEA-expressing HCT116 cells. Furthermore, mAb-heparin and anti-CEA mAb-gelonin conjugates were found to mainly localize on the cell membrane with minimal internalization. In contrast, cells incubated with TAT-gelonin/mAb-heparin complex showed significant uptake enhancement that increases with longer incubation time. In xenograft tumor bearing mice, enhanced and localized delivery of the complex was observed in the tumor, with ~60-fold increase of complex accumulation in the tumor site as compared to the uncomplexed conjugates and inhibited tumor growth by 46%. Overall, this study demonstrated the strategy of combining the target specificity of mAb and the membrane permeability of PTD for intracelluar delivery of highly potent toxins for colorectal cancer therapy.

Conjugation Effects on Antibody−Drug Conjugates: Evaluation of Interaction Kinetics in Real Time on Living Cells An antibody modified with a small molecule cargo may affect the antibody interaction with the target and alter

its association rate and/or dissociation rates. Real time detection of the binding of ADCs to its antigen allows one to probe if the kinetic profile has changed as a consequence of conjugation. Björkelund and colleagues at Uppsala University describe a method for monitoring changes in binding kinetic properties of antibodies conjugated with small molecules by detecting antibody−antigen interaction in real time using the instrument LigandTracer and the interactions of cetuximab with EGFR and AbD15171 with CD44v6. Measurements were performed using cells that express membrane-bound antigens. It was found that conjugation of Texas Red dye to lysines on cetuximab led to reduced interaction with EGFR and slower association and dissociation rates. In contrast, 125I modification on tyrosine residues rendered cetuximab to bind EGFR with a significantly faster association rate, reaching equilibrium within 3.5 h, and had a larger impact on the off rate with about half of the antibody dissociated over the first 8 h. Overall, 125I reduced the affinity of the cetuximab−EGFR interaction by one order of magnitude. Meanwhile, neither Texas Red nor 125I impacted the interactions of AbD15171 and CD44v6. These findings demonstrate that a cargo can impact the kinetics and affinity of an antibody to its target, and that this effect may differ between antibodies and conjugation type. The changes may affect the efficacy and safety of the ADC and the real time profile of altered, or unaltered, binding pattern of the conjugated antibody can lead to favorable improvements and selection of desirable conjugation strategy.

Phage selection of bicyclic peptides binding Her2 Aberrant expression of the EGFR Her2 is implicated in various malignancies including breast cancer. The mAbs trastuzumab and pertuzumab and the antibody-drug conjugate (ADC) trastuzumab emtansine, which target Her2, are widely used in the clinics. While antibodies dramatically improve treatment outcomes of Her2-positive patients, the efficacy of these proteins in targeting tumors is still limited due to their large size of ~150 KDa and their unequal distribution in solid tumors. The discovery that the Her2-targeting hexapeptide KCCYSL, identified from a phage display library, suggests that Her2-specific peptide with diverse scaffolding can be developed. Diedrich and Heinis from Ecole Polytechnique Fédérale de Lausanne developed Her2-specifc ligands based on peptides that are two-orders of magnitude smaller than mAbs. Peptides have advantages over proteins in ADC discovery because of ease of synthesis and conjugation, higher molecule-per-mass ratios, and anticipated better tumor penetration. They used a phage panning strategy to display bicyclic peptides and selected 30 low micromolar to high nanomolar binders against the extracellular domain of Her2. The best bicyclic peptide, identified after affinity maturation, bound Her2 with a KD of 304 nM that is comparable to KCCYSL (KD = 295 nM). Although the binding affinities of the best peptide ligand candidate is likely not very sufficient for further testing in in vitro models, additional iterative cycles of mutagenesis and affinity maturation can further enhance its affinity to Her2.

3. Profile of a Young Scientist

Dr. David Rabuka is currently the Global Head of R&D at Catalent Pharma Solutions. In 1996, he obtained his BS with double honors in chemistry and biochemistry from the University of Saskatchewan in Saskatoon. In 1999 he earned his MS in chemistry from the University of Alberta, studying carbohydrate chemistry under the direction of Ole Hindsgaul. He went on to work at the Burnham Institute, where he synthesized complex glycans. Next, he was the employed at Optimer Pharmaceuticals, where he focused on the development of glycan- and macrolide-based antibiotics. He obtained his Ph.D. in chemistry and chemical biology from the University of California, Berkeley, as a Chevron Fellow. His graduate work in Carolyn Bertozzi's lab focused on developing chemical tools for studying glycoproteins. He has authored 35 papers and 7 patents.

Dr. Rabuka's current research is focused on the development of antibody-drug conjugates. Following graduate school, he founded Redwood Bioscience Inc., which licensed an aldehyde-tagging technology developed and patented by the Bertozzi Lab. Utilizing the bioorthogonal chemistry methods developed in the Bertozzi Lab, Redwood Bioscience is applying the aldehyde-tag technology to develop site-specific antibody drug conjugates. In this approach, an antibody amino acid sequence is mutated to include a five amino acid sequence tag (CXPXR) that is recognized by a formylglycine-generating enzyme, which converts the cysteine into an aldehyde tag. This aldehyde tag is then used as a chemical handle for attachment of a cytotoxic drug molecule. Since the enzyme only recognizes the specific recognition sequence, antibody-drug conjugates can be developed with specific drug-to-antibody ratios and defined sites of attachment. Recently, Redwood Bioscience was acquired by Catalent Pharma Solutions and David continues to further develop the aldehyde tag technology and explore novel biotherapeutics at Catalent as the Global Head of R&D, Chemical Biology.

4. Spotlight on World News

Google announces Nanoparticle Diagnostic Research Google X laboratories announced the development of nanoparticle diagnostics paired with a wearable detector at the Wall Street Journal's WSJD Live conference on Oct. 28, 2014. Google X envisions designing functionalized nanoparticles to bind to something specific, such as circulating tumor cells. Ideally, the nanoparticles would be swallowed in pill form and enter the bloodstream. The particles' tiny cores could

be made from magnetic iron oxide, a compound already in Food & Drug Administration-approved nanoparticle contrast agents. A wearable device that creates a magnetic field could summon the particles from outside the body.

Myriad Loses DNA Patent Case Appeals court says tests for breast and ovarian cancer genes are not patent-eligible. A federal appeals court has rejected a bid by Myriad Genetics to block sales of competing DNA-based tests for determining hereditary risk for developing breast and ovarian cancer. Claiming patent infringement, Myriad had sought to prevent Ambry from marketing a test for the BRCA1 and BRCA2 genes, which are linked to breast and ovarian cancer.

New Drug Approvals soar in 2014 In an encouraging sign of the health of the pharmaceutical industry, new drug approvals soared to an 18-year high in 2014. The Food & Drug Administration gave the green light to 41 new molecular entities last year, up from 27 in 2013. The 2014 class of new drugs is ripe with innovation: The list includes 16 first-in-class treatments, compared with just nine drugs with a novel mechanism of action approved in 2013. Last year was the best year ever for rare disease drug approvals, FDA said, with orphan drugs accounting for roughly 40% of the new drugs approved. Other highlights were 12 new treatments for infectious diseases, including four new antibiotics. Eight new cancer drugs hit the market, with several immunotherapies that represent major advances for patients. Seven of those drugs were approved by the FDA in the last month of the year.

Pfizer and Merck Form Immunotherapy Coalition Pfizer will pay the German drugmaker Merck KGaA $850 million plus up to $2 billion more in milestones to codevelop MSB0010718C, a Merck anti-PD-L1 antibody in clinical trials as a cancer treatment.

Evotec and Sanofi partner on early-stage drug development Evotec AG and Sanofi have begun exclusive negotiations for a major multi-component strategic alliance focused on early-stage drug development that could run over the next five years. This collaboration will entail a minimum guaranteed commitment from Sanofi to Evotec of €250 million over the next five years, including an upfront cash payment to be defined in the agreement. The initiative will also include a co-development agreement with associated upfront, development, regulatory and sales milestones, in addition to royalties that will benefit both parties.

Evotec and Ohio State University collaborate on novel cancer therapy Evotec AG, has announced a research collaboration with the laboratories of Prof. Roger Briesewitz at The Ohio State University Comprehensive Cancer Center, Arthur G. James Cancer Hospital and Richard J. Solove Research Institute. The objective of the collaboration is to progress a novel mechanism for engaging the KRas target discovered at The Ohio State University using Evotec's technology platform and broad expertise in drug discovery and pre-clinical development, to validate and progress novel leads into pharmaceutically developable candidates.

KRas is a small GTPase regulating the RAS/MAPK signalling cascade that governs cell division. Mutations in KRas are early transforming events in tumorigenesis and are highly prevalent in lethal cancers such as lung, colon and pancreatic cancer. Addressing the challenging tractability of this well-established oncology target, work conducted at Ohio State provides a novel route to engage the target.

5. Career Forum

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

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

http://jobs.rsc.org/

http://chemistryjobs.acs.org/

6. Conferences

American Association for Cancer Research Annual Meeting 2015
April 18-22, 2015, Philadelphia, Pennsylvania, USA

US Human Proteome Organization – Next Generation Proteomics
March 15-18, 2015, Tempe, AZ

DNA Methylation
March 29 – April 3, 2015, Keystone, CO, USA

Epigenetic Inhibitor Discovery
April 21-22, 2015, San Diego, CA, USA

American Society of Clinical Oncology (ASCO) Annual Meeting
May 29-June 2, 2015, Chicago, Illinois, USA

63rd American Society for Mass Spectrometry on Mass Spectrometry and Allied Topics
May 31-June 4, 2015, St. Louis, MO

AACR Precision Medicine Series: Integrating Clinical Genomics and Cancer Therapy
June 13 - 16, 2015 Salt Lake City, Utah

Targeting Histone Acetylation
June 11-12, 2015, Boston, MA, USA

The 5th Conference on Notch targeting in cancer
June 24-26, 2015, Mykonos, Greece.

Epigenetics, Chromatin, and Transcription
June 28 – July 3, 2015, West Palm Beach, FL

Helicases and Nucleic-Acid Based Mechanisms: From Mechanism and Insight to Disease
July 26-31, 2015, Steamboat Springs, CO

Histone Deacetylases and Sirtuins in Biology, Disease, and Aging
August 16-21, 2015, Timmendorfer Strand, Germany

14th Human Proteome Organization World Congress
September 27-30, 2015, Vancouver, Canada

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
November 5 - 9, 2015 Boston, Massachusetts