Major Research Interests and Accomplishments:

1. Translational Pharmacology Studies of Nanoparticle, Carrier Mediated Agents (CMA), and Biologics

Significance and Innovation. The research program I lead is among the world's leading laboratories in the translational development of complex agents, such as carrier-mediated agents (CMAs), nanoparticles, and biologics, and the pharmacologic and phenotypic characterization of these agents. We have developed novel analytical and pharmacologic methods to characterize the complex pharmacokinetic disposition of the conjugated and released forms of these agents in blood, tumor, and tissues. We have used this extensive experience and novel methods to perform our own groundbreaking pharmacology studies of nanoparticle agents and collaborate on the development of nanoparticle agents from investigators in the Carolina Center for Cancer Nanotechnology Excellence (CCCNE), Carolina Institute for Nanomedicine (CIN), UNC ESOP Center for Nanotechnology in Drug Delivery, NCI Nano Alliance, the U.S. FDA, other academic institutions, and numerous pharmaceutical companies. This work has produced numerous funded grants and contracts and publications as highlighted in the section on my CV.

Impact and Future Directions. The PK of these agents is complex, and detailed studies must be performed to evaluate the disposition of the carrier-associated or encapsulated form of the drug and the released form (**Fig 1**). We have developed unique and detailed methods to evaluate the PK of nanoparticle encapsulated and released drug in plasma for several carrier-mediated agents, including Doxil. In addition, the variability in the function and phenotype appears to be a primary driver and/or rate limiting step that affects the systemic clearance and biodistribution on CMAs in preclinical models and in patients. Thus, development of biomarkers of the mononuclear phagocyte system (MPS) may be a novel technology and methodology to address the high and complex variability in the PK and PD of nanoparticle, carrier mediated agents, and biologics.

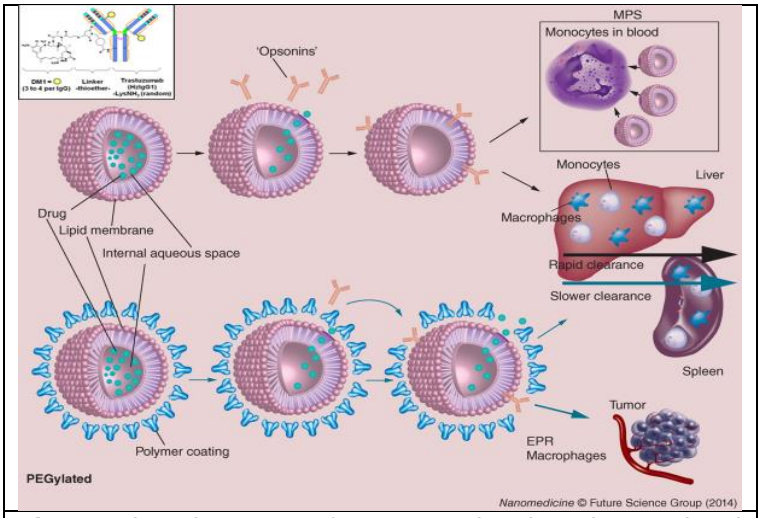

Fig 1. The clearance of non-PEGylated and PEGylated nanoparticles, antibodies, and antibody drug conjugates (ADCs) via the mononuclear phagocyte system (MPS) in blood, liver, and spleen.

Notable Publications.

1. Price LSL, Stern ST, Deal AM, Kabanov AV, **Zamboni WC**. A Reanalysis of Nanoparticle Tumor Delivery Using Classical Pharmacokinetic Metrics. Science Advances. Science Advances 15 Jul 2020: Vol. 6, no. 29, eaay9249. DOI: 10.1126/sciadv.aay9249
2. Caron WP, Morgan KP, Zamboni BA, **Zamboni WC**. A review of study designs and outcomes of phase I clinical studies of nanoparticle agents compared with small molecule anticancer agents. Clinical Cancer Res. 2013;19(12):3309-15. PMID: 23620407.
3. **Zamboni WC**, Torchilin V, Patri A, Hrkach J, Lee R, Stern S, Nel A, Malghan S, Panaro N, Grodzinski P. Best Practices in Cancer Nanotechnology: Perspectives from NCI Nanotechnology Alliance. Clinical Cancer Research. Clin Cancer Res. 18(12);3229-41:2012. PubMed Central: PMC3916007.
4. **Zamboni WC,** Maruca L, Strychor S, Zamboni BA, Ramalingam S, Friedland DM, Edwards RP, Stoller RG, Belani CP, Ramanathan RK. Pharmacokinetic study of pegylated liposomal CKD-602 (S-CKD602) in patients with solid tumors. Clinical Pharmacol Ther. 86(5);519-26:2010. PubMed PMCID: PMC3428134.

2. Biomarkers for the Bi-Directional Interaction between Carrier-Mediated Agents (CMA) and the Innate Immune System (IIS) / Mononuclear Phagocyte System (MPS)

Significance and Innovation. My research program is also the first group to develop biomarkers of the mononuclear phagocyte system (MPS), which is part of the innate immune system (IIS), as a platform to

characterize CMAs based on their interaction with the MPS and to individualize nanoparticle therapy (**Fig 2**). We have published information on the relationship between MPS function in blood, hormone, and chemokine mediators of the IIS/MPS in patients and the effects of IIS/MPS in preclinical models on the PK and PD of CMAs. In addition, we have developed a high throughput screening platform to profile the interaction between CMAs and biologics, and the MPS. These novel methods were used to perform our own ground-breaking pharmacology studies of CMAs and collaborate on the development of numerous CMAs.

Impact and Future Directions. Our goal is to translate the biomarkers of the IIS/MPS into clinical practice in order to create new paradigms and platforms to evaluate the PK and PD of CMAs. In addition, these biomarkers can be used to optimize and individualize the dose and treatment regimen for CMAs in patients in general and in special patient populations(e.g. geriatric patients and obese patients). As there are >200 CMA agents approved and in development our studies may have a far-reaching impact.

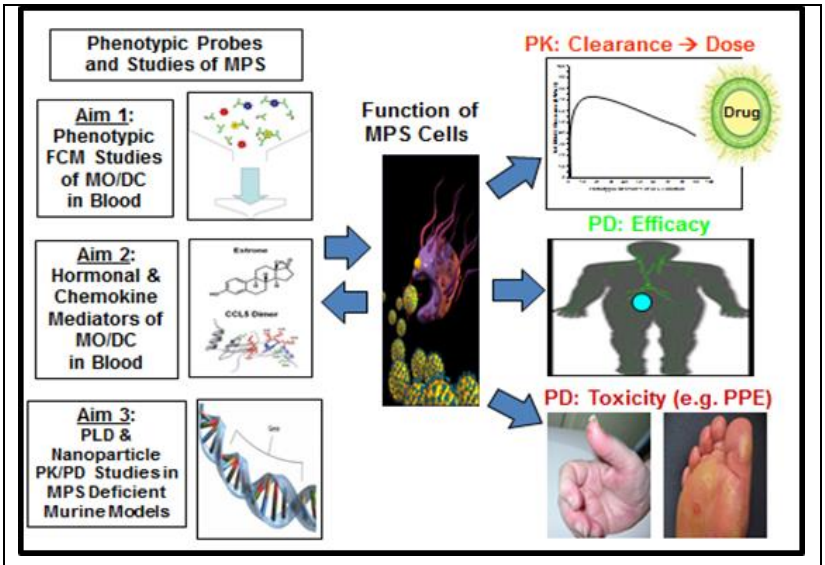

Fig 2. The anticipated relationship between cellular phenotypic probes, mediators and genetic factors affecting the MPS and the PK and PD of PLD.

Notable Publications.

1. Giovinazzo H*, Kumar P*, Sheik A, Ivanovic M, Walsh M*, Caron WP*, Kowalsky RJ, Song G*, Whitlow A, Clarke-Pearson DL, Brewster WR, Le LV, Bae-Jump V, Gehrig PA, **Zamboni WC**. Technetium-99m-Sulfur-Colloid as a Phenotypic Probe for the Pharmacokinetic and Pharmacodynamic disposition of PEGylated Liposomal Doxorubicin in Women with Recurrent Epithelial Ovarian Cancer. Cancer Chemother Pharmacol. 2016;77(3):565-73. PMID: 26822231.
2. Song G, Suzuki O, Santos CM, Wiltshire T, **Zamboni WC**. Gulp1 is associated with pharmacokinetics of PEGylated liposomal doxorubicin (PLD) in inbred mice strains. Nanomedicine. 2016;12(7):207-2017. PMID: 27288666.
3. Yang Q, Jones SW, Parker CL, **Zamboni WC**, Bear JE, Lai SK. Evading immune cells uptake and clearance requires PEG grafting at densities substantially exceeding the minimum for brush conformation. Mol. Pharmaceutics. 2014; 11(4): 1250-1258. PubMed PMID: 24521246.
4. Caron WP, Lay JC, Fong AM, La-Beck NM, Kumar P, Newman SE, Zamboni BA, Crona DJ, Clarke-Pearson DL, Brewster WR, Le LV, Bae-Jump V, Gehrig PA, **Zamboni WC**. Translational studies of phenotypic probes of the mononuclear phagocyte system and nanosomal pharmacology. J of Pharmacol Exp Ther. 2013;347(3):599-606. PMID: 24042160.

3. Biomarkers for the Relationship between the Innate Immune System (IIS) / Mononuclear Phagocyte System (MPS) and Pharmacokinetics (PK) and Pharmacodynamics (PD) of Monoclonal Antibodies (mAbs) and Antibody Drug Conjugates (ADCs)

Significance and Innovation. An objective of the Precision Medicine Initiative, recently launched by the U.S. FDA and NIH, is to optimize and individualize dosing of drugs, especially anticancer agents, with high pharmacokinetic (PK) and pharmacodynamic (PD) variability and low therapeutic index when used alone and especially in combination with other agents. Carrier-mediated agents (CMAs), such as nanoparticles (NPs), monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs) are agents that specifically fit this objective. Compared to small molecule drugs, NPs, mAbs and ADCs are cleared via the cells of mononuclear phagocyte system (MPS), also called antigen-presenting cells, and is part of the innate immune system (IIS). Variability in MPS function and chemokines mediators (CCL2 and CCL5) have been shown to predict variability in the pharmacokinetics (PK) and pharmacodynamics (PD) of NPs such as PEGylated liposomal doxorubicin (Doxil®; PLD) in patients and preclinical models. In addition, circulating MPS cells, such as peripheral blood mononuclear cells (PBMCs), are a depot site for NP exposures. While NPs are cleared via phagocytosis by MPS cells, the MPS also serves as a natural mechanism of clearance for antibodies and immune complexes via their Fc-gamma-receptors (FcγR) on MPS cells (**Fig 3**). Myeloid cells express various forms of FcγRs (CD64, CD32, CD16) that will interact with extracellular monomeric or aggregated IgGs and therapeutic mAbs and ADCs. FcγRs are also expressed on non-MPS cells, such as neutrophils, T-cells, and B-cells. Due to the differences in types and affinity in FcγRs, variations in receptor expression can lead to significant differences in the ability of MPS cells and potentially non-MPS cells to clear immune complexes from the blood. This translates to variability in the ability of MPS and non-MPS cells to take up mAbs and ADCs, which would affect their PK and PD. The high PK variability is clinically important for mAbs, and especially ADCs, as these agents have a narrow therapeutic index. The combination of ADCs with other mAbs may increase the likelihood of drug-drug interactions and altered PK and PD of the ADC as these agents are also cleared by and may alter the MPS. Thus, MPS biomarker studies are critically important to optimizing the treatment of mAbs and ADCs alone and in combination. *Our overall hypothesis is that mAbs and ADCs are cleared via the FcγRs on MPS and biomarkers of FcγRs and chemokines can be used to evaluate patient-specific differences in PK and PD of these agents and risk for drug-drug interactions, especially for regimens combining ADCs and mAbs.*

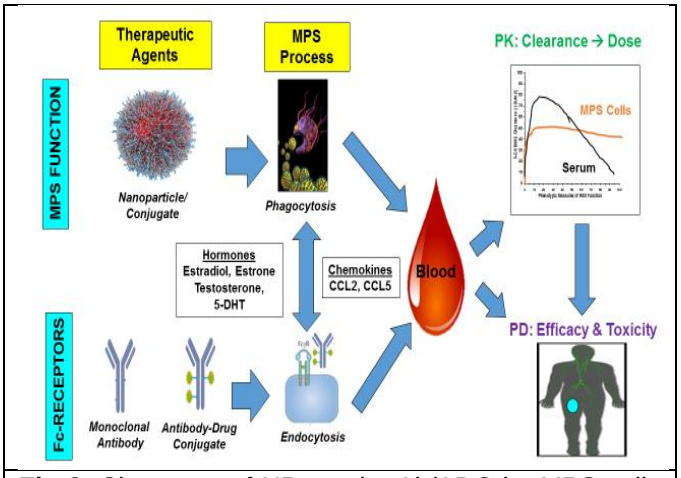

Fig 3. Clearance of NPs and mAb/ADC by MPS cells via phagocytosis and FcγR endocytosis, respectively.

Impact and Future Directions. Our goal is to translate the results of these studies into clinical practice in order to create new paradigms and platforms to evaluate the PK and PD of mAb and ADCs. As there are >200 mAb and ADC agents approved and in development our studies may have a far-reaching impact. Used to individual dose or mAbs and especially immuno-oncology mAbs in patients. These will be a major focus on my ongoing and future research. These biomarkers of the IIS/MPS are currently being evaluated in 3 clinical trials funded by the NCI and 3 other preclinical studies funded by grants and contracts. The evaluation of biomarkers of the IIS/MPS to optimize the dose of mAbs, especially immuno-oncology mAbs, and ADCs is a current major focus of my research.

Notable Publications.

1. Lucas AT*, Robinson R, Schorzman AN, Piscitelli J*, Razo J*, **Zamboni WC**. Pharmacologic considerations in the disposition of antibodies and antibody-drug conjugates in preclinical models and in patients. Antibodies. 2019; 8(1): 3. doi: 10.3390/antib8010003. PMID: 31544809.
2. Lucas AT, Price LSL*, Schorzman AN, Storrie M*, Piscitelli JA*, Juan Razo, **Zamboni WC**. "Factors affecting the pharmacology of antibody-drug conjugates". Antibodies. 2018; 7(1): 10. doi: 10.3390/antib7010010. PMID: 31544862.
3. Kirschbrown WP, Lucas AT, **Zamboni WC**, Garg A. Biomarkers of Fc-gamma receptors (FcγRs) on Mononuclear Phagocyte System (MPS) Cells in Blood of Patients with Advanced Gastric Cancer are upregulated as compared to Patients with Metastatic Breast Cancer. EORTC-NCI-AACR Conference 2018.
4. Lucas AT*, Herity LB*, Kornblum ZA, Madden AJ*, Gabizon A, Layko D, Kabanov AV, Ajamie T, Bender DM, Kulanthaivel P, Sanchez-Felix MV, Havel HA, **Zamboni WC**. Pharmacokinetic and screening studies

of the interaction between mononuclear phagocyte system and nanoparticle formulations and colloid forming drugs. Int J Pharm. 2017;526(1-2):443-454. PMID: 28473237.

5. This evaluation of biomarkers for the relationship between the IIS/MPS and the PK and PD of monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs) is a major focus of my current and future research. For example, we are currently in the processing of generating three separate publications on this topic (n=2 clinical studies; n=1 preclinical study).

4. Profiling and Modulating Factors that Inhibit the Tumor Delivery of Carrier-Mediated Agents (CMAs) and Biologics

Significance and Innovation. The research program I lead also is among the leaders in profiling and modulating the tumor delivery of nanoparticle and carrier-mediated agents (CMAs). The theoretical advantages of CMAs in cancer treatment include increased solubility, prolonged duration of exposure, selective delivery of entrapped drug to the tumor, and an improved therapeutic index. The promise of these drugs is unfulfilled due to an overall low tumor uptake. In theory, enhancing permeability of the tumor vasculature allows CMAs to enter the tumor interstitial space, while suppressed lymphatic filtration allows them to stay there. This phenomenon, termed the Enhanced Permeability and Retention (EPR) effect, may be exploited by CMAs to deliver drugs to tumors. However, progress in developing effective CMAs using this approach has been hampered by heterogeneity of EPR effect in different tumors and the lack of information on factors that influence EPR. In addition, cancer cells in tumors are surrounded by a complex microenvironment comprised of endothelial cells of the blood and lymphatic circulation, stromal fibroblasts, collagen, cells of the mononuclear phagocyte system (MPS) and other immune cells that may be associated with the variability in EPR and are potential barriers to tumor delivery of CMAs (**Fig 4A**). Moreover, it appears that the ability of CMAs to enter tumors by EPR or other factors is highly variable across tumor types and thus all solid tumors may not be conducive for CMA delivery and treatment. Thus, it is important to development new methods to overcome barriers and increase the tumor delivery of several different types of CMAs in solid tumors with different degrees of EPR effect.

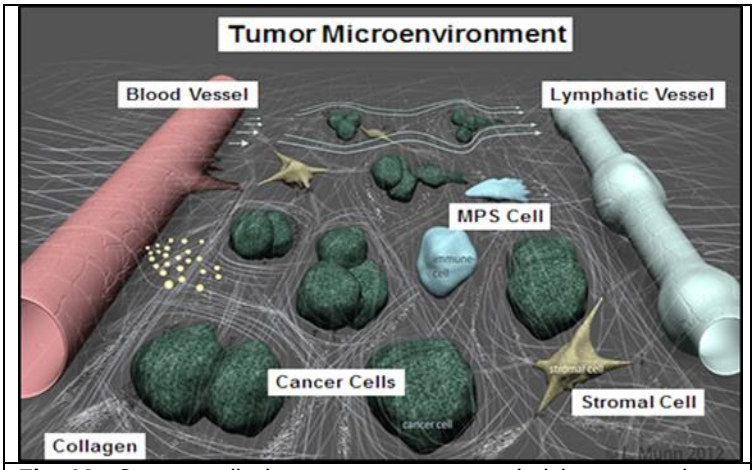

Fig 4A. Cancer cells in tumors are surrounded by a complex microenvironment comprised of numerous cells including blood vessels, pericytes and lymphatics, stromal cells, collagen and MPS cells that may alter NP delivery. We will profile these tumor related factors and evaluate how they are altered by MRT and CRT and this affects the tumor delivery of CMAs.

In theory, the enhanced permeation and retention effect **(**EPR) exists in tumors and may be exploited for selective delivery of drugs to tumor by CMAs. However, PK studies show that in reality the tumor delivery of CMAs is low and inefficient due to tumor heterogeneity and associated barriers. We conducted a meta-analysis on the plasma and tumor PK of CMAs and small molecule (SM) anticancer agents using standard PK parameters and a novel PK metric called relative distribution index over time (RDI-OT) which measures the efficiency of tumor delivery. The RDI-OT versus time profiles of CMAs and SMs in tumor are depicted in **Fig 4B**. The lower efficiency of tumor delivery seen with CMAs compared with SMs (blue lines) suggests there may be an inherent limit to CMA entry into tumors. Thus, the development of new methods to overcome these barriers and increase the delivery of CMAs to tumors is critically needed. We have identified several novel methods to increase the tumor delivery of CMAs, including small molecule and NP formulations o agents that alter inflammatory cells and the use of the novel

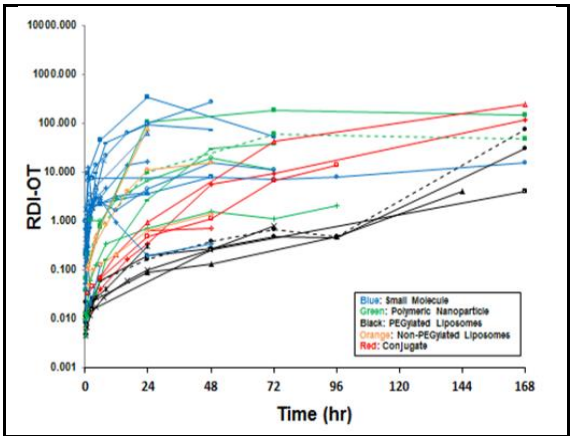

Fig 4B. RDI-OT versus time profiles of CMAs and SMs in tumor. Agents are SMs (blue), polymeric NPs (green), PEG-liposomes (black), non-PEG-liposomes (orange) and NP-conjugates (red). SMs have the highest efficiency of tumor delivery as depicted by the blue lines.

microbeam radiation therapy (MRT).

Impact and Future Directions. Based on our groundbreaking work on MRT+PLD in a breast cancer GEMM, we will evaluate the ability of MRT to enhance the tumor delivery and efficacy of Onivyde and PL-SN38 in murine models of pancreatic cancer, which has even greater barriers to tumor delivery than breast cancer. This work will be funded by an NIH RO1 grant with myself as the Lead-PI. As Onivyde is FDA approved for the treatment of pancreatic cancer and human and large scale MRT treatments have already been used clinically (e.g. GRID therapy and Lattice therapy), if the proposed preclinical studies are successful, the results may lead to a phase 1/2 clinical study MRT-SFRT + NP as pre-surgical neoadjuvant treatment in patients with locally advanced pancreatic cancer. This proposal aims to overcome the inherent major barriers in NP delivery to tumors and safely increase the drug delivery and efficacy of these agents in pancreatic cancer and other solid tumors. Because there are >200 NPs currently in development this proposal may have a far-reaching impact.

Notable Publications.

1. Price LSL, Stern ST, Deal AM, Kabanov AV, **Zamboni WC**. A Reanalysis of Nanoparticle Tumor Delivery Using Classical Pharmacokinetic Metrics. Science Advances. Science Advances 15 Jul 2020: Vol. 6, no. 29, eaay9249. DOI: 10.1126/sciadv.aay9249.
2. Song G, Darr DB, Santos CM, Ross M, Valdivia A, Jordan JL, Midkiff BR, Cohen S, Feinberg, Miller CR, Tarrant TK, Rogers AB, Dudley AC, Perou CM, **Zamboni WC**. Effects of tumor microenvironment on nanoparticle disposition and efficacy in triple negative breast cancer. Clinical Cancer Res, 2014; 20(23):6083-95. PMID: 25231403.
3. Chu KS, Hasan W, Rawal S, Walsh MD, Enlow EM, Luft JC, Bridges AS, Coleman J, Napier ME, **Zamboni WC**, DeSimone JM. Plasma, tumor and tissue pharmacokinetics of docetaxel delivered via nanoparticles of different sizes and shapes in mice bearing SKOV-3 human ovarian xenograft. Nanomedicine. 2013;9(5):686-93. PMID: 23219874. NIHMSID: NIHMS433583.
4. Prabhakar U, Maeda H, Jain R, Sevick-Muraca E, **Zamboni W**, Barry S, Gabizon A, Grodzinski P, Blakey D. Challenges and key considerations of the enhanced permeability and retention effect (EPR) and nanomedicine drug delivery in oncology. Cancer Research. 2013;73(8):2412-7. PubMed Central: PMC3916009.
5. **Zamboni WC**, Eiseman JL, Strychor S, Rice PM, Joseph E, Zamboni BA, Donnelly MK, Shurer J, Parise RA, Tonda ME, Yu NY, Engber C, Basse PH. Tumor disposition of pegylated liposomal CKD-602 (S-CKD602) and the reticuloendothelial system in preclinical tumor models. J of Liposome Research 21(1);70-80:2010. PubMed PMID: 20528623.

5. Detection and Removal of Surface Exposures of Hazardous Drugs in Hospitals and Pharmacies.

Significance and Innovation. Antineoplastic agents are known to be harmful to both healthy and cancerous cells, and thus are considered hazardous drugs. The new U.S. Pharmacopeia (USP) Chapter 800 outlines standards to protect health care personnel when handling hazardous drugs. One strategy to minimize exposure to health care employees is to ensure that the environment has minimal contamination of antineoplastic agents on surfaces. USP Chapter 800 recommends that environmental wipe sampling for hazardous drug surface residue be performed routinely (e.g., initially as a benchmark and at least every 6 months, or more often as needed) to verify containments. As related to this recommendation, Dr. Stephen Eckel from UNC ESOP and I developed products to detect (sample kit) and remove (HDClean™) hazardous drugs from surfaces in hospitals and pharmacies. These technologies were then licensed from UNC to a company we created called ChemoGLO, LLC (**Fig 5**). Dr. Eckel and I serve as scientific advisors for ChemoGLO. ChemoGLO services and research involves a group of professional individuals who are passionate about the impacts of hazardous drugs on patients and healthcare workers.

Impact and Future Directions. Using ChemoGLO to identify areas of contamination will help institutions evaluate the risk of harmful exposure to hazardous drugs. In addition, using HDClean as part of a regular cleaning program will help reduce the risk of exposure to employees and patients alike. ChemoGLO is also developing additional novel products for the detection and removal of hazardous drugs alone and in collaborations with investigators at UNC and other academic centers and companies. These products and research have resulted in ChemoGLO being a leader in this field and the most frequently used hazardous drug testing kit and reference lab in the world. The UNC ATPAC Lab performs samples analyses and, research and development studies for ChemoGLO via UNC Office of Sponsored Research approved service agreements and contracts.

Fig 5. ChemoGLO surface sampling kit (left) and HDClean cleaning towelettes (right).

Notable Publications and Information.

1. Salch SA, **Zamboni WC**, Zamboni BA, Eckel SF. Patterns and characteristics associated with the surface contamination of hazardous drugs in hospital pharmacies. Am J Health Syst Pharm. 2019 Apr 17;76(9):591-598. PMID: 31361828.
2. Cox J, Speed V, Hasselwander T, O'Neil S, Sherwood C*, Eckel S, **Zamboni WC**. Development and evaluation of a novel product to remove surface contamination of hazardous drugs. J Oncol Pharm Pract. 2017;23(7):558-560. PMID: 28791909.
3. **Zamboni WC**, Salch SA*, Cox J, Eckel S. It takes a village to raise awareness of and to address surface contamination of hazardous drugs. J Oncol Pharm Pract. 2017;23(7):558-560. PMID: 28791909.
4. www.chemoglo.com