RESEARCH STRATEGY: Multiscale Personalized Cancer Vaccines for Treating and Preventing Metastatic Breast Cancer

Introduction. Breast cancer remains the second leading cause of cancer death among women. With primary breast cancer largely manageable by surgical resection, chemotherapy, and radiotherapy, metastatic breast cancer (MBC) remains a major threat and is resistant to most of conventional therapies.[1-3] To date, there are still no effective therapies to cure or prevent MBC, motivating the development of new therapies for treating MBC and ideally preventing the onset of MBC. Immunotherapy has shifted the paradigm for cancer treatment in the past two decades, especially with the success of checkpoint blockade and chimeric antigen receptor (CAR) T cell therapy.[4-9] Indeed, the combination of anti-PD-1 and chemotherapy has been approved for treating recurrent PD-L1-expressing MBC, albeit with a modest therapeutic benefit.[10-13] CAR T cell therapies that target Her2 and MUC1 are also explored for MBC treatment in clinical trials.[14-18] While showing limited clinical success at the moment, cancer vaccines are undeniably one of the most promising modalities for both treating and preventing cancer, by inducing the presentation of cancer antigens on antigen presenting cells (e.g., dendritic cells (DCs)) in the body to generate persistent tumor-specific T cell response and humoral response.[19-22] The power of vaccines is well demonstrated by the success of HPV vaccine for lowering the risk of cervical, penile, anal, vaginal, and head and neck cancers.[23-28] In the context of breast cancer without a clear association to viruses, though, developing potent and safe cancer vaccines remains challenging. To date, there are still no effective vaccines for treating or preventing MBC, despite the extensive preclinical efforts in developing DNA vaccine, peptide vaccine, mRNA vaccine, tumor lysate vaccine, tumor exosome vaccine, and nanomaterial vaccine and translational efforts led by various cancer centers, the Artemis Project, Metavivor, and others.[29-34]

Vision. I envision that future MBC vaccines should accommodate four different scenarios: (**i**) MBC is diagnosed at the same time as the diagnosis of primary breast cancer (which accounts for ~6% of women breast cancer patients[35]), (**ii**) MBC is detected as a metastatic recurrence post surgical resection of primary breast cancer (which accounts for 20-30% of patients with primary breast cancer[36-38]), (**iii**) preventive measure for patients diagnosed with primary breast cancer but not MBC, and (**iv**) preventive measure for women *not* diagnosed with breast cancer. In my opinion, the first success in MBC vaccine would come at the treatment of MBC in scenario (**i**), by exploiting the primary breast tumor to identify MBC-associated antigens and compose therapeutic MBC vaccines. If successful, MBC vaccines developed through scenario (**i**) will naturally extend to scenario (**ii**). The success of MBC vaccines in treating MBC for these scenarios would be crucial to boost the confidence of patients and clinicians to pursue the prophylactic arm of MBC vaccines in scenarios (**iii**) and (**iv**). **My vision for therapeutic MBC vaccine is that a multi-scale cancer vaccine platform, which fully exploits the different forms of antigens to accommodate MBC heterogeneity and the urgency of different MBC scenarios, is needed to potentially lead to clinical breakthroughs for treating MBC** (**Fig. 1**).

(1) Multiscale cancer vaccines for treating MBC (scenarios **i** & **ii**). Therapeutic cancer vaccines aim to enrich and stimulate tumor-specific $CD8^+$ T cells to better combat MBC. Two key components are needed for developing an effective MBC vaccine: tumor antigen and DC-activating adjuvant. Among the different sources of tumor antigens, neoantigens that result from genetic mutation of tumor cells have the highest specificity, but are limited by the lengthy and costly identification process (3-4 weeks) and low abundance on tumor cells.[39-42] Tumor cell lysates[43-45] or tumor-derived extracellular vesicles (EVs)[46-48] cover a broader spectrum of tumor antigens, including those abundant epitopes, and only take several days to fabricate a vaccine, but suffer from the low specificity. ***Race***

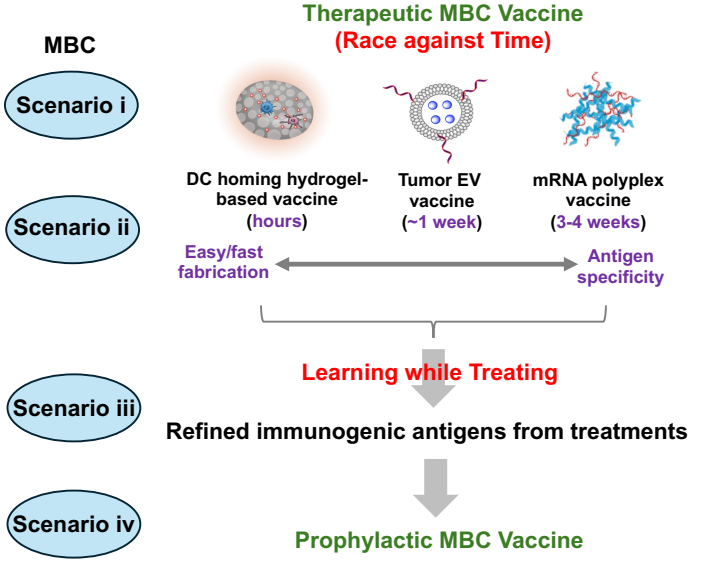

Figure 1. Development of multiscale cancer vaccines for treating and preventing four different scenarios of MBC. We integrate DC homing hydrogel-based vaccine, tumor extracellular vesicle (EV) vaccine, and mRNA polyplex vaccine that can be fabricated from days to weeks to cover varied urgency of MBC. With a 'learning while treating' concept, we will build a refined database of MBC antigens that show potent $CD8^+$ T cell response and antitumor efficacy.

against time: The time needed to fabricate clinical-grade MBC vaccines is a critical factor in clinical settings. To this end, my lab has aimed to establish cancer vaccine platforms that cover the time range from hours (DC-homing hydrogel based cancer vaccine) to days (EV vaccine) to weeks (neoantigen mRNA vaccine). *The DC-homing hydrogel-based cancer vaccine* is composed of a macroporous hydrogel encapsulating DC-attracting chemokines and tumor cell lysates. Upon subcutaneous injection, DCs can be actively recruited into the gel and present tumor antigens, before they migrate to draining lymph nodes to prime antigen-specific $CD8^+$ T cells.[49-52] The hydrogel can be pre-fabricated and used as an off-the-shelf product. *Our EV-based cancer vaccine* is composed of a conjugate of tumor EV and DC-activating adjuvant (e.g., CpG). This is based on our recently developed technology that can metabolically introduce >3,000 chemical tags (e.g., azido groups) to the surface of each tumor EV.[53,54] The chemical tags enable the conjugation of a tunable amount of adjuvants onto tumor EVs for optimal modulation of DCs and amplification of antitumor efficacy.[53,54] *Our neoantigen mRNA vaccine* is composed of the polyplex of neoantigen-encoding mRNA and α-helical polypeptide. The α-helical polypeptide can stabilize mRNA, facilitate the cellular uptake of mRNA by temporally disrupting DC membrane, and meanwhile activate DCs in a timely manner. Two doses of polyplexes resulted in 33.3% tumor-free survival against 4T1 TNBC, in contrast to 0% for lipoplexes or mRNA-loaded lipid nanoparticles. The rate-limiting step of developing neoantigen mRNA vaccines, though, is the lengthy process of identifying neoantigens via DNA/RNA sequencing, epitope prediction, and *in vitro* screening, which takes 3-4 weeks. **Through this Era of Hope award, I aim to address the key challenges associated with the development of robust DC homing hydrogel vaccine, EV vaccine, and mRNA vaccine for treating MBC, and fully integrate these cancer vaccine platforms to cover the varied urgency of breast cancer patients, towards finding a long-term solution for MBC treatment**.

(2) Cancer vaccines for preventing MBC (scenarios **iii** & **iv**). The main challenge for developing a prophylactic MBC vaccine lies in the identification of immunogenic MBC-associated antigens.[55-58] For scenario **iii**, primary breast tumor can be exploited to identify antigens that are potentially shared by the emerging MBC. For scenario **iv**, a database of antigen candidates is needed to identify potential MBC antigens. Different from conventional top-down approaches that give a large library of antigen candidates from genome sequencing, **my vision for developing robust prophylactic MBC vaccine emphasizes the importance of finding the most promising immunogenic antigens from therapeutic vaccine studies (Fig. 1)**. This 'learning while treating' concept aligns with the urgency of MBC, increases the likelihood of success in identifying more effective epitopes, and builds up the confidence of patients in trying the developed prophylactic MBC vaccines.

Accomplishments. Since starting my lab in August 2020, I have been focused on making progress in finding a cure for breast cancer and building leadership in cancer research across and beyond the campus. I have made several key advances in cancer immunotherapy that build on my past work yet open the possibilities for the ambitious future program I propose here, yielding 15 research articles and 9 review articles with me as the corresponding author and 8 patent applications. I have also established collaboration with clinicians at Carle Hospital, OSF Healthcare, and MD Anderson Cancer Institute to push forward the clinical translation of several immunotherapies developed in my lab. *Awards*: I have secured an NIH NCI R01 grant (2022), a perfect-score NIH NCI R21 grant (2023), NSF CAREER award (2021), CDMRP Idea Development Award (2024), CMBE New Innovator award (2023), Scialog Fellow (2023), attendee for the Grainger Foundation Frontiers of Engineering Program by National Academy of Engineering (2023), Dean's Award for Excellence in Research (2024), American Cancer Society Research Scholar Award (2024), and Sontag Distinguished Scientist Award (2024). I was also nominated by the Campus Awards and Honors Committee for the National "Innovators in Science Award". As highlighted below, I have a track record of developing innovative cancer and immune cell targeting technologies and further developing clinically translatable targeted therapies and immunotherapies, which I expect to carry on to address the pressing needs of a cure for MBC.

1) Cancer-selective metabolic labeling and targeting. Among the first to apply metabolic glycan labeling and click chemistry for *in vivo* cancer-targeted delivery of therapeutics, I demonstrated that the treatment of tumors with azido-sugars can mediate superior targeting of dibenzocyclooctyne (DBCO)-bearing agents via click chemistry with >500% targeting effects. To achieve cancer-selective labeling, I designed trigger-responsive azido-sugars that can selectively label cells with clickable azido groups only in the presence of a specific trigger (ultraviolet light, hydrogen peroxide, enzymes, etc.).[59] One of them, a cathepsin L/histone deacetylase-responsive azido-sugar, that can selectively label tumors *in vivo* for subsequent targeted delivery of therapeutics.[59]

Connection to breast cancer and MBC: This new cancer-targeting technology can be applied to cancers that cannot be targeted by conventional antibody strategies, such as TNBC that lacks characteristic biomarkers, and has resulted in a startup company (IRIA Pharma) to pursue clinical translation.

2) In vivo labeling and targeting of dendritic cells (DCs). A facile strategy to target DCs in the lymph nodes is critical for generating persistent effector T cell responses, but was lacking in the field. I developed a technology that, for the first time, enables *in vivo* metabolic labeling and targeting of DCs[51]. Our technology can yield azido-tagged DCs in the lymph nodes where they can capture antigens, adjuvants, and cytokines via click chemistry. *Connection to breast cancer and MBC*: We demonstrated the promise of the DC-targeted immunotherapies for treating TNBC in murine models,[55,60,61] with the clinical translation being actively pursued through the Dana Farber Cancer Institute and Wyss Institute.

3) In situ antigen-free breast cancer vaccines. The identification of tumor neoantigens is a costly and time-consuming process and the rarely identified neoantigens are often poorly immunogenic. In one notable work, I developed a macroporous hydrogel-based *in situ* cancer vaccine that can release chemotherapeutics to induce the generation of endogenous antigens form tumor cells while recruiting high numbers of DCs *in situ*. The recruited DCs can present the *in situ* generated tumor antigens, for subsequent priming of tumor-specific $CD8^+$ T cells[50]. *Connection to breast cancer and MBC*: This *in situ* cancer vaccine shows potent efficacy against TNBC and provides a personalized and universal vaccination platform that can bypass the antigen identification steps.

4) Rationale design of immune cell homing materials. In contrast with conventional immunotherapy that relies on inefficient trafficking of injected immunomodulators to lymphatic tissues, I have developed immune cell homing materials that can actively recruit and modulate immune cells *in situ*. For example, macroporous hydrogels loaded with GM-CSF and tumor antigens, upon subcutaneous injection, can recruit and modulate DCs *in situ* for subsequent priming of tumor-specific T cells[51]. My lab has developed macroporous hydrogels with independently tunable pore size and mechanics, and elucidated the impact of each parameter on the immune cell homing profile. *Connection to breast cancer and MBC*: Breast cancer antigens, including tumor lysates, EVs, and neoantigen-coding mRNAs, can be loaded into gels to compose a breast cancer vaccine.

5) An EV metabolic tagging and targeting technology. My group has developed a facile metabolic tagging technology that can install >3,000 azido groups (versus 1-100 for conventional modification methods) onto the surface of each tumor EV.[53] The surface azido groups enable conjugation of sufficient molecules via click chemistry for the tracking and modulation of EVs. We demonstrated that the conjugation of toll-like receptor 9 agonists onto EVs enables timely activation of DCs and generation of superior antitumor $CD8^+$ T cell responses. *Connection to breast cancer and MBC*: Our study yields a universal technology to generate chemically tagged EVs from breast cancer cells, modulate EV-cell interactions, and develop potent EV-based breast cancer vaccines.

6) Invention, Intellectual Property and Translation. Although early in my career, I have already filed 12 invention disclosures and patents. One of them has led to the formation of a start-up company named IRIA Pharma. **Patents Issued or Pending**: (1) Cheng J, **Wang H**. Trigger-activatable metabolic sugar precursors for cancer-selective labeling and targeting. U.S. Patent No. 11,014,953. (Start-up company: IRIA Pharma). (2) Mooney D, **Wang H**. Compositions and methods for labeling and modulation of cells in vitro and in vivo. U.S. Patent No. 17/206,050. (3) Najibi A, Shah N, **Wang H**, Mooney D. Biomaterials-based antigen free vaccine and the use thereof. U.S. Patent No. 62/904,446. (4) **Wang H**, Khalil A, Mooney D, Jaenisch R. Methods for labeling and targeting cells. U.S. Patent No. 62/967,387. (5) **Wang H**, Bhatta R, Han J. Modified exosomes and methods of use. U.S. Patent No. 63/333,001. (6) Liu Y, Mooney D, **Wang H**. Compositions and methods for localized delivery of cytokines for adoptive cell therapy. U.S. Patent No. 63/330,562. (7) **Wang H**, Liu Y. Metabolic tagging and targeting of red blood cells. U.S. Patent No. 63/402,413. (8) **Wang H**, Bo Y. Methods and kits for reducing the risk of allograft rejection. U.S. Patent No. 63/550,246. (9) Wang C, Zhao Z, Zhang X, **Wang H**, Han J. Wireless magnetic robot for mechanotherapy. U.S. Patent No. 63/638,410. (10) **Wang H**, Bhatta R. Injectable extracellular vesicle hydrogels and the use thereof. U.S. Patent No. 63/692,788. (11) **Wang H**, Han J. Self-adjuvanting α-helical polypeptides for mRNA cancer vaccines. U.S. Patent No. 63/680,442. (12) Anti-RNA for the treatment of type-1 diabetes and autoimmune diseases. UIUC-2025-056. **Moving fundamental scientific discoveries to clinical impact**: I have been involved in several translation projects in the area of targeted cancer therapy and cancer immunotherapy. Through IRIA Pharma, we have been testing the feasibility of labeling breast cancer with clickable tags, for subsequent targeted delivery of chemotherapeutics or radioisotopes via click chemistry.[59] Through Wyss Institute and Dana Farber Cancer Institute, we are testing the safety profile of DC-targeted cancer

vaccines in human patients, based on the DC labeling and targeting technology described above.[51] Here, at the University of Illinois, my lab is collaborating with oncologists at Carle Hospital, OSF HealthCare, and MD Anderson Cancer Center to pursue clinical translation of several cancer immunotherapies, including a biomaterial scaffold-based cancer vaccine for treating breast cancer and a tumor EV vaccine for treating glioblastoma and breast cancer. **In summary, I have a strong track-record of innovation, high-impact studies, research productivity, and translational efforts**.

Leadership. I firmly believe that it is not sufficient for faculty researchers today to only make key scientific breakthroughs. I have also devoted myself to contributing to the scientific community as a scientist, mentor and volunteer. *Scientifically*, I am an active member of AACR, BMES, ACS, MRS, SFB, AIChE, CRS, and AAPS, and organized and chaired various symposia in research conferences. At the Cancer Center at Illinois (CCIL), I co-initiated the Immunology working group in July 2022 to bring together research labs for active collaboration in cancer immunotherapy. During 2023-2024, I was selected (as the only Assistant Professor) to participate in the competitive Leadership Program offered by the Institute for Genomic Biology at UIUC. In Fall 2024, I was selected as one of four Scholars to participate in the Faculty Leadership Development Program which aims to foster leaders and develops a plan for succession within CCIL. Currently, I am the Scientific Lead of the Shared Resources at CCIL and oversee the strategic plan of research use/needs at CCIL. *As a mentor and volunteer*, I serve in the Cancer Research Advocacy Group (CRAG) at UIUC to regularly meet with breast cancer patients and advocates to update breast cancer research landscape and promising clinical trials, and discuss pressing needs of breast cancer patients. Five breast cancer patients and advocates visited my lab and had a round-table chat with my trainees and colleagues in July 2024. I have been actively communicating with breast cancer advocates on social media, invited two breast cancer patients to our 2024 CCIL annual retreat, and had a gathering with a group of breast cancer patients/advocates at 2025 AACR meeting in Chicago. Our department also entrusted me to host mid-GLAM since summer 2022, which is a week-long summer camp to teach middle school girls the definition, structure, properties, and applications of materials. It is so gratifying to see so many girls get excited about our biomaterial-based breast cancer vaccines. Recently, my lab has started to work with breast patient advocates and clinicians at Carle Health, OSF Healthcare, and Siteman Cancer Center at St Louis to prepare research posters and promote breast cancer research in local schools and on social media. *Future*: As an Era of Hope Scholar, I will lead cutting edge research whilst being an ambassador for DoD BCRP. In particular, I will continue to support and engage with breast cancer patients and advocates, lead and organize meetings and workshops for breast cancer researchers and patients through CRAG, CCIL, and symposia/conferences, lead and expand the immunology working group, initiate a new breast cancer research working group at UIUC, and integrate breast cancer research in educational and outreach activities including our annual Engineering open house and mid-GLAM. My lab will continue to host on-site visits of breast cancer patients and advocates, and actively promote breast cancer research through research presentations and social media posts. I am also committed to bringing together researchers in academia, clinic, and industry via symposia and conferences to foster open discussions and efforts on the translation of breast cancer vaccines and immunotherapies, and work together with patient advocates to raise the awareness of the importance of developing cancer vaccines as a long-term solution for breast cancer.

Research Goals. The primary goal of our project is to develop robust cancer vaccines for treating and preventing MBC. Combating MBC is a race against time and resources, and it is impossible to win the race with one single vaccine platform. Our strategy, with a 'learning while treating' rationale, integrates multiple vaccine platforms (i.e., DC homing hydrogel-based vaccine, EV vaccine, and mRNA vaccine) that can be fabricated from days to weeks to cover all scenarios of MBC. We will address the key challenges barring the antitumor efficacy of each vaccine platform (Aim 1-3), and build a database of immunogenic human breast cancer antigens from the therapeutic studies. Compared to existing deep sequencing and machine learning methods that provide a large library of untested antigen candidates,[62-65] our refined database of antigens that already demonstrate favorable CTL responses and therapeutic efficacy will increase the likelihood of success to find the right antigens and develop off-the-shelf MBC vaccines (Aim 4).

1) DC-homing material-based MBC vaccines. In contrast with conventional immunotherapies that rely on inefficient trafficking of injected immunomodulators to lymphatic tissues, we will utilize DC-homing materials that can actively recruit and program high numbers of DCs *in situ*.[49-52] One key ***challenge*** for developing a robust

DC homing material-based MBC vaccine is the ability to recruit a purer population of DCs. My lab has recently uncovered the impact of material properties (mechanics and pore size) on the immune cell homing profile and developed a macroporous alginate hydrogel system that can recruit >85% DCs. Breast cancer cells can be lysed and loaded into the pre-fabricated GM-CSF-loaded macroporous alginate gel to compose the cancer vaccine within **minutes**. Upon subcutaneous injection of the gel vaccine, DCs will be recruited to the gel, take up and present tumor antigens, and migrate to draining lymph nodes to prime antigen-specific T cells (**Fig. 2**). The dose of tumor lysate, gel volume, and necessity of incorporating a DC-activating adjuvant will be optimized for CTL response and antitumor efficacy against two widely used MBC models: 1) FVB/N-Tg(MMTV-PyVT)634Mul/J mice with spontaneous palpable mammary tumors that metastasize to lungs[66-68] and 2) Balb/c mice i.v. injected with 4T1 TNBC[50,69,70]. The gel vaccine will be administered before or after the establishment of MBCs to evaluate the prophylactic and therapeutic efficacy, respectively. Immune cells in the blood and tumor, especially tumor-specific T cells, will be analyzed. Human-version gel vaccines will be fabricated by lysing surgically resected or biopsied human breast cancer cells and loading them into the pore-forming alginate gel, and subcutaneously injected into $CD34^+$ humanized NSG mice[71-75] with established MBC (via i.v. injection of human breast cancer cells), to assess the therapeutic efficacy. To study the ability of the gel vaccine to prevent human MBC, gel vaccines will be subcutaneously injected into $CD34^+$ humanized NSG mice first, followed by i.v. injection of human breast cancer cells. Neoantigen-specific T cell clones that are enriched in the blood and tumor will be identified and added to our database of immunogenic human breast cancer neoantigens.

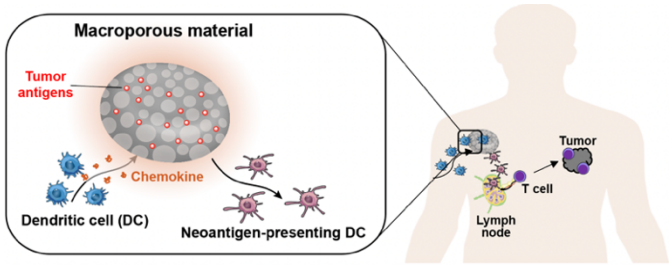

Figure 2. Chemokine-releasing macroporous materials for *in situ* recruitment and modulation of DCs, for subsequent priming of tumor-specific effector T cells in the draining lymph nodes.

2) EV vaccines for treating and preventing MBC. Tumor EVs are regarded as a safer source of tumor antigens than tumor lysates, but the modest therapeutic efficacy of existing EV vaccines remains a major limitation. Previous efforts to simply mix EVs and adjuvants have also largely failed to improve the antitumor efficacy. We recently developed a technology to metabolically label tumor EVs with chemical tags (e.g., >3,000 azido groups per EV), which enables conjugation of sufficient and controllable amounts of immunomodulators onto the surface of EVs (**Fig. 3a**).[53] As nanosized EVs enter DCs via endosomes where Toll-like receptor 9 (TLR9) exists, we further demonstrated that the conjugation of sufficient CpG (a TLR9 agonist) to tumor EVs dramatically improved the activation of DCs compared to the mixture of CpG and EVs (>175-fold effect) (**Fig. 3b**).[53] We envision our EV labeling technology and the enabled EV vaccine shows great promise for treating and preventing MBC. We will fabricate EV-based MBC vaccine by culturing surgically resected or biopsied breast cancer cells with azido-sugars for **3-7 days**, isolating azido-tagged EVs (10^8-10^9), and conjugating with TLR9 agonists. The ability of EV vaccines to elicit robust CTL response and antitumor efficacy will be tested in the above-described murine MBC models and human MBC xenograft model. The enriched human neoantigen-specific T cell clones in the blood and tumor will be identified and added to our breast cancer neoantigen database.

3) mRNA vaccines for treating and preventing MBC. To generate a potent CTL response, mRNA vaccines should (i) induce efficient uptake and processing of mRNAs by DCs, (ii) properly activate DCs to facilitate the presentation of neoantigens via major histocompatibility complexes (MHC), and (iii) further allow the programmed DCs to access $CD8^+$ T cells. To this end, mRNA-loaded lipid nanoparticles (LNPs) have demonstrated pronounced success in humoral immunity-dominated COVID-19 vaccines, but are not as effective for cancer treatment which

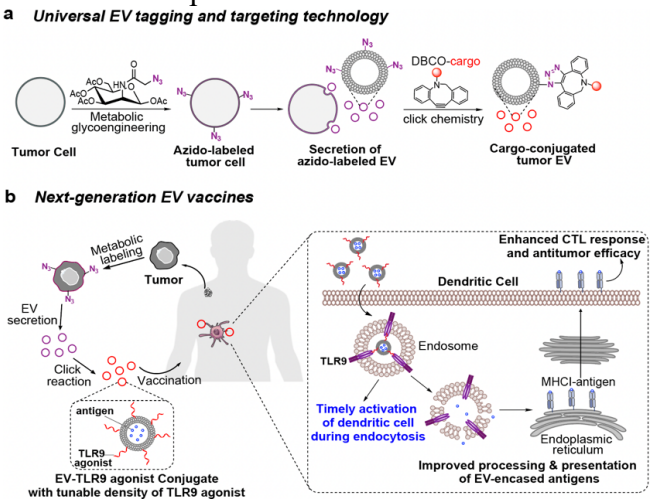

Figure 3. Metabolic tagging and targeting of tumor EVs for developing robust EV vaccine. (**a**) Tumor cells can be metabolically labeled with azido groups, for secretion of azido-tagged EVs that enable conjugation of DBCO-cargo. (**b**) CpG-conjugated EVs can be internalized by DCs via endosomes. The binding of CpG on EVs to TLR9 on endosomes can stimulate DCs timely, leading to improved presentation of EV-encased antigens.

demands more vigorous T cell activation. My lab recently developed a class of self-adjuvanting α-helical polypeptides that serve as both an mRNA carrier and a DC-activating adjuvant, leading to significantly improved antitumor efficacy of neoantigen mRNA vaccines against solid tumors (**Fig. 4**). In this Aim, we will elucidate the mechanisms underlying the polypeptide-mediated DC activation and screen for the best-performing polypeptide for mRNA cancer vaccines. We will identify neoantigens from surgically resected or biopsied human breast cancer cells, and synthesize neoantigen-encoding mRNAs and their complexes with α-helical polypeptides. The mRNA polyplex vaccine will be assessed for therapeutic and prophylactic efficacy against human MBC in CD34$^+$ humanized NSG mice. This set of experiments will also inform the types of human breast cancer neoantigens that can induce potent CTL responses and antitumor efficacy.

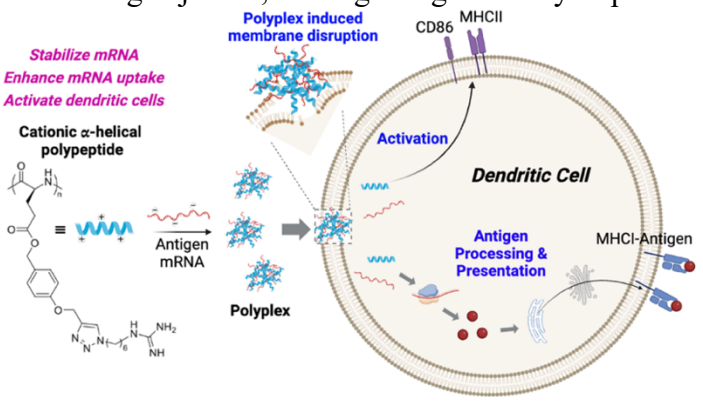

Figure 4. Self-adjuvanting *α*-helical polypeptides for simultaneous mRNA delivery and DC activation. *α*-helical polypeptide can stabilize neoantigen mRNAs and facilitate intracellular delivery of mRNAs via its cell-penetrating property. Also, *α*-helical polypeptide can provide a 'danger signal' to activate DCs and improve neoantigen presentation.

4) Towards the development of off-the-shelf prophylactic MBC vaccines. One ultimate goal is to develop off-the-shelf vaccines that can prevent MBC. Our strategy is to build a database for immunogenic antigens that show potent CTL response and therapeutic efficacy in Aim 1-3 studies. Ideally, antigens or antigen-encoded mRNAs from this refined database can be synthesized and stored as an off-the-shelf product. When in need of a prophylactic MBC vaccine, mRNA polyplex vaccine and DC homing hydrogel-based vaccine can be rapidly fabricated. As a proof-of-concept demonstration, we will compare the CTL response and prophylactic efficacy of MBC vaccines using human breast cancer antigens in our refined database versus those in conventional database.

Research Barriers: I regard this Era of Hope Scholar Award as a unique opportunity to lay out my imagined future of MBC vaccines. Each of the above-described research aims could lead to independent breast cancer vaccines for potential clinical translation, and as a whole, I dream of a future when multiscale personalized cancer vaccines are available for treating and preventing different scenarios of MBC. Main research barriers include i) optimization of each cancer vaccine system, ii) elucidation of chronic side effects associated with each cancer vaccine, and iii) interpretation of the animal study data regarding applicability towards human settings. For i), we have established each vaccine platform in the lab, and have refined the key component for optimization (e.g., type of adjuvant for EV vaccine, combination of neoantigens for mRNA vaccine, and choice of mechanics and pore size for the gel vaccine). For ii), we have included pathologists and immunologists in our team to understand and address potential long-term side effects of cancer vaccines. For iii), we have proposed the assessment of human-version vaccines in humanized mouse models in this proposal. As CD34$^+$ humanized NSG mice (the best model available) do not fully mimic the human immune system, I have initiated the collaboration with the organoid team (funded by an ARPA-H grant) at UIUC to assess cancer vaccines in patient-derived immune cell-incorporated breast cancer organoids. Another major challenge would be the alignment of our ambitious initiative with the clinical practice. To mitigate this challenge, I have established multiple collaborations with oncologists in Carle Hospitals, OSF Healthcare, Siteman Cancer Center at St Louis, and MD Anderson Cancer Center to evaluate the safety and effectiveness of the biomaterial-based cancer vaccine, EV vaccine, and mRNA polyplex vaccine systems against melanoma, glioblastoma, and renal cancer, as a means to de-risk the clinical development of each vaccine system. I am also constructing new collaborations with breast cancer surgeons to gain more insights into the clinical practice of breast cancer treatment, and adapt our cancer vaccine systems to different scenarios of breast cancer in the clinic. **Commitment**: I will dedicate at least 2.25 months (25%) of effort specifically to this project. More efforts will be devoted to the in-depth mechanistic understanding of the cancer vaccine systems in parallel projects. **Research Environment**: UIUC has a strong track record of equipment and facility support, interdisciplinary collaborations, and translation of research discoveries. I am an affiliate of seven Departments/Units on campus, and my lab members have access to all the resources needed for this project. We have established collaborations with a number of research groups across and beyond the campus, including 7 clinicians, over the past 4.5 years. **In summary, I propose a multi-scale personalized cancer vaccine approach that covers different stages and urgency of breast cancer, for the treatment and prevention of MBC**.