7th Annual
Immune Modulation and Engineering Symposium

Trainee Awards

CD45-targeted HIV Env trimers produce robust immunity with a single shot | *Award for Collaborative Research*

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A successful HIV vaccine will require the induction of broadly neutralizing antibodies (bnAbs) targeting conserved regions of the variable HIV Env antigen. Because bnAbs from HIV+ individuals undergo extensive somatic hypermutation (SHM), vaccines must drive sustained germinal center (GC) activity where SHM occurs. One strategy to support this is directing antigens to lymph node follicles using nanobody-based targeting. We hypothesized that fusing nanobodies recognizing immune cell surface markers to HIV Env trimers could enhance immune responses by improving antigen trafficking, retention, and GC persistence.

To test this, BG18-class HIV Env trimers were engineered with C-terminal VHH fusions (Env-VHHs) targeting CD45, MHC-II, or GFP (control). These were expressed in HEK293 cells, purified, and validated for structural integrity by chromatography and TEM, antigenicity by ELISA, and targeting specificity by ELISA and flow cytometry. In vivo, CD45-targeted trimers showed significantly higher uptake by B cells, T cells, NK cells, neutrophils, and dendritic cells 24 hours post-injection compared to controls. Immunization studies in mice demonstrated that CD45-targeted Env-VHHs induced faster seroconversion, larger GCs, and more Env-specific GC B cells 21 days post-prime than untargeted or control-targeted trimers. A single dose of CD45-targeted vaccine also elicited strong, durable serum antibody and memory B cell responses that matched or exceeded those seen with boosted untargeted trimers.

These results suggest that targeting Env to immune cell surfaces—particularly CD45—enhances both the magnitude and quality of adaptive immune responses. By improving antigen delivery to secondary lymphoid tissues, this approach may offer a promising strategy to overcome current challenges in HIV vaccine design."

IL-33-releasing hydrogels for treating ischemic muscle injuries| *Award for Innovative Research*

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Peripheral artery disease (PAD)—characterized by stenosis of arteries supplying blood to the extremities—is a leading cause of morbidity worldwide that can result in functional decline and auto-amputation of limbs. Emerging therapeutics to revascularize occluded vessels are marked by limited efficacy due to their short half-life and poor retention in the target ischemic tissue. These approaches are further impaired by prolonged pro-inflammatory responses associated with PAD, resulting in dysfunctional regeneration and fibrosis. Recent evidence suggests that infiltrating regulatory T cells (Tregs), which are widely recognized for mitigating inflammation, are critical for neovascularization, muscle repair, and tissue remodeling following injury. However, the persistent pro-inflammatory microenvironment of severe injuries limits Treg recruitment and regenerative potential. As such, we engineered an injectable alginate hydrogel for the sustained release of interleukin(IL)-33, a chemoattractant for Tregs, to enhance Treg-mediated repair following ischemic injury. Incorporation of charged laponite nanodiscs into the hydrogel permitted enhanced burst release and long-term rate of IL-33 release by modulating electrostatic interactions between the drug and hydrogel. Delivery of IL-33-

releasing hydrogels in a murine model of hindlimb ischemia (in BALB/c mice) demonstrated enhanced blood perfusion recovery to 60% of basal levels in their ischemic hindlimbs relative to administration of a blank hydrogel, which recovered only 30% of basal perfusion. These trends aligned with the progression of necrosis in the hindlimb following injury; blank hydrogel-treated mice exhibited a rapid onset of necrosis—resulting in auto-amputation of the toes, while delivery of IL-33 hydrogels attenuated these outcomes to mild digit discoloration. Furthermore, functional assessment of the ischemic tibialis anterior muscle yielded increased average in situ twitch and tetanic contractile forces following delivery of IL-33-releasing hydrogels compared to blank hydrogels. Overall, this study provides preliminary in vivo data to support the use of injectable, Treg-targeting biomaterials as a potential strategy to treat ischemic injuries.

A Lipo-Polymeric Nanoparticle Drug and Gene Delivery Platform for Enhanced Immune Responses Against Melanoma | *Award for Innovative Research*

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In the last few years, and especially during the COVID-19 pandemic, gene therapy has gained tremendous momentum due to the rapid emergence and large-scale commercial production of lipid nanoparticle (LNP) mRNA vaccines. Despite the achievements of the COVID-19 LNP mRNA vaccines, serious challenges remain regarding their stability, limiting the extent of their distribution and leading to widespread vaccine wastage. While polymeric NPs have been proposed as an alternative, lack of efficacy and concerns about toxicity have largely limited their development. This research aims to develop a novel polymer-lipid hybrid NP platform that employs surface loading of mRNA and can be lyophilized into a stable powder to increase the shelf life of vaccines that utilize these NPs as nucleic acid delivery devices. Furthermore, these NPs can be used to encapsulate therapeutic drugs thus enabling the development of dual therapy approaches in which genetic material and drugs can be co-delivered simultaneously using a single NP delivery system. This work demonstrates that these NPs can be used to safely and efficiently transfect cells with both RNA and DNA, achieving equivalent transfection efficiency to that of conventional LNPs. Both water-soluble and organic-soluble drugs can be loaded into these NPs and delivered alongside transfection, demonstrating an enhancement to therapeutic effects in systems designed to provoke inflammatory immune responses. In a B16F10 tumor model in mice we demonstrated that dual intratumoral delivery of trametinib- and GM-CSF mRNA-loaded NPs resulted in three times slower tumor growth and improved median survival times by 32% compared to untreated tumor-bearing mice. Overall, this work provides evidence for the potential of these NPs to not only overcome current challenges associated with mRNA vaccine stability but also unlock new dimensions of vaccine design through combination delivery of mRNA and drugs to produce synergistic therapeutic effects.

‘Goldilocks’ Thermal Window as Defined by Theranostic PET for Optimal Focused Ultrasound Thermal Ablation and CD47 Antagonist Delivery | *Award for Translational Research*

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Background: Focused ultrasound (FUS) is rapidly redefining non-invasive oncology by depositing precise, non-ionizing acoustic energy deep within solid tumors. In the thermally ablative regime (T-FUS), it can simultaneously cytoreduce tumor burden and ignite local immune activation, making it an attractive backbone for antibody-based immunotherapies. Yet, therapeutic success depends on hitting a “Goldilocks” thermal window: too little heat fails to provoke immunogenic remodeling, while excessive ablation collapses the vascular pathways antibodies needed for tumor entry. To quantify this balance, we utilize a theranostic immunoPET strategy to chart the spatiotemporal kinetics of a model tumor-targeted checkpoint antibody (αCD47) following T-FUS.

Methods: 4T1-tumor bearing mice underwent T-FUS with a custom ultrasound-guided FUS system (Fig.1A). Shear wave elastography (SWE) was serially performed before and after T-FUS using a

Philips EPIQ scanner. Following T-FUS, mice received intravenous injection of [18F]-Fludeoxyglucose (FDG), followed one day with later injection of [89Zr]-αCD47 and serial PET/CT imaging. Terminal ex-vivo biodistribution analysis was performed and quantified via automated gamma counter.

Results: T-FUS induced an acute reduction in tumor stiffness, as measured by SWE, which normalized to sham levels within 2 days—suggesting a transient relaxation of tissue stiffness that may define a therapeutic window for enhanced antibody delivery prior to microenvironmental reconstitution (Fig.1B-C). FDG-PET scans revealing a significant reduction in tumor metabolic activity acutely following ablation (Fig.1D-F). [89Zr]-αCD47 PET confirmed antibody uptake in T-FUS tumors, with only modest post-ablative reduction and gradual recovery by day 3 post-ablation (Fig.1G-H). At this time point, ex-vivo biodistribution confirmed reduction, but not deletion, of intratumoral antibody access in the T-FUS group (Fig.1I-J), with unchanged peripheral organ distribution (Fig.1K-P).

Conclusions: A theranostic PET approach revealed that T-FUS can impact but is not deleterious to tumor-targeted antibody penetrance, herein demonstrated with [89Zr]-αCD47—provoking future consideration of windows for optimal tumor-drug exposure in T-FUS paradigms. Future studies will expand findings to other solid tumor and immunotherapy settings.

In Vitro Assessment of Hydrogel-Coated Cardiovascular Shunt Hemocompatibility for Pediatric Applications | *Award for Leadership in Diversity*

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Congenital heart defects are a leading cause of infant mortality worldwide. In the most severe cases, infants are born with one ventricle severely underdeveloped. Life-saving heart reconstruction surgery is performed, most often including implantation of a fixed-diameter cardiovascular shunt to divert blood flow from the aorta to the lungs. A major limitation is that fixed-diameter shunts cannot accommodate rapid infant growth; to overcome this, we have developed a geometrically-tunable hydrogel-coated cardiovascular shunt. This innovative design allows the shunt to adjust its diameter through light exposure. Upon implantation, any medical device in contact with blood interacts with various cells in the blood and vasculature. As such, cyto- and hemo-compatibility is essential to device translation. Dextran, a polysaccharide commonly used for its biocompatibility and high-water solubility, is utilized as a polymer backbone to attach photoresponsive methacrylate groups by glycidyl methacrylate (50% substitution). Cylindrical hydrogels (10%w/v) were formed by crosslinking with dithiothreitol (DTT; DTT/methacrylate ratio 20-50%). Cytotoxicity (3T3 fibroblasts and human umbilical vein endothelial cells (HUVECs), Cell Counting Kit-8), inflammatory response (RAW-Blue macrophage reporter cell line), hemolysis (ASTM 756-13), platelet activation/aggregation (flow cytometry: CD62p, forward scatter), and complementary activation (C3a ELISA) are investigated to establish state-of-the-art methods for comprehensive evaluation of material hemocompatibility in vitro. These investigations show that 3T3 fibroblasts and HUVECs exhibit population doubling times consistent with published data and comparable to untreated controls. RAW-Blue macrophages displayed minimal adhesion to hydrogel surfaces; pro-inflammatory activation was comparable to negative polystyrene controls. Hemolysis was comparable to negative controls (LDPE, PTFE) and within ASTM and ISO standards. Platelet activation/aggregation and complementary activation showed no significant difference between hydrogel and negative controls. Collectively, the size-tunable shunt developed addresses current clinical limitations in pediatric care, and cytocompatibility of the hydrogels used in the device development suggests their exceptional suitability for diverse blood contacting applications.

HTLV-infection derived exosome-mediated transcriptional reprogramming in Monocytes of HAM/TSP patients | *Award for Leadership in Diversity*

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Human T-lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic neuroinflammatory disorder often leading to demyelination of the spinal cord. Progression to HAM/TSP is closely associated with the high viral load and the presence of virally infected CD4+ T cells that release extracellular vesicles (EVs). Exosomes, a type of EV secreted by many cell types, carry proteins and nucleic acids that modulate cell-to-cell communication and have been implicated in cancer and neuroinflammatory disease progression. Herein, we have studied the effect of exosomes from HTLV-1 infected cells onto the PBMCs of HAM/TSP patients by single cell sequencing utilizing innovative HoneyComb technology. We observed a unique response in monocyte populations compared to other cell types. Interestingly, monocytes are the least studied cell types in HTLV-1 pathogenesis, and therefore this observation provided a brand-new avenue to understand HAM/TSP pathogenesis especially with respect to the influence of infection-derived exosomes on immune dysregulation. A total of 41 genes were identified to be differentially expressed in HAM/TSP monocytes treated with exosomes; 28 were upregulated and 13 were downregulated. From these, we focused on genes with the most significant expressional changes: CXCL5, PPBP, IL-7R, INHBA, and CSF-1 were upregulated, while FUCA1, APOC1, APOE, CCL2, and CTSD were downregulated. These results are currently being validated. CXCL5, PPBP, and CCL2 are associated with chemokine activity, indicating altered immune cell recruitment. IL-7R, INHBA, and CSF-1 are involved in cytokine-mediated signaling, with IL-7R also playing a role in T-cell differentiation. APOC1 and APOE are linked to lipid transport and macrophage functions. Downregulation of FUCA1 and CTSD, both involved in lysosomal and glycoprotein degradation, may reflect impaired antigen processing. Overall our data suggest that exosome-treated HAM/TSP monocytes undergo immune remodeling that favors cell recruitment, activation, and a shift toward an M2-like immunoregulatory phenotype. Such a shift may support viral persistence and chronic inflammation. These findings highlight a potential therapeutic pathway for addressing HTLV-1-induced neuroinflammation by modulating exosome-mediated signaling.

Poster Presentations

1. Biomaterial Scaffolds for In Vivo Generation of CAR T Cells

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Chimeric antigen receptor (CAR) T cell therapy is paradoxical, delivering unprecedented clinical success while remaining largely inaccessible due to its ~$0.5 million cost per dose and >6-week manufacturing time. These barriers stem from the complex ex vivo processes currently required for CAR T cell production, which also negatively affect their functionality. An emerging solution is in vivo CAR T cell manufacturing, in which CAR T cells are generated directly within the patient, bypassing ex vivo manipulation and potentially improving efficacy.

Biomaterials offer a powerful platform for enabling in vivo CAR T cell therapies by providing tunable, biocompatible environments that can facilitate T cell recruitment, activation, transduction, and expansion. Our lab has developed Multifunctional Alginate Scaffolds for T Cell Engineering and Release (MASTER)-implantable scaffolds that reduce CAR T cell manufacturing to a single day from blood collection to administration. In this system, peripheral blood mononuclear cells isolated from the patient are co-seeded with CAR-encoding virus onto the scaffold, which is then implanted subcutaneously. Embedded anti-CD3/CD28 antibodies and IL-2 stimulate the T cells, which are transduced in the scaffold and subsequently released into circulation. In a mouse xenograft lymphoma model, MASTER-generated CAR T cells showed superior phenotype and persistence compared to conventional CAR T cells.

Here, we advance the MASTER platform to generate CAR T cells entirely in vivo in a syngeneic mouse model by incorporating T cell-recruiting chemokines into the scaffolds that recruit endogenous T cells. The recruited T cells can then be activated, transduced, and expanded in the scaffold to produce and release functional CAR T cells fully in vivo, bypassing the ex vivo cell isolation and manipulation required in the MASTER system. By further streamlining the CAR T cell therapy process, this approach offers the potential to dramatically reduce manufacturing complexity, expand patient access, and improve efficacy.

2. Engineering Rac-Enhanced CAR-Macrophages (Race-CAR-M) to Improve Solid Tumor Immunotherapy

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Triple-Negative Breast Cancer (TNBC) is an aggressive subtype lacking estrogen, progesterone, and HER2 receptors, which limits targeted therapy options. Although Chimeric Antigen Receptor T-cell (CAR-T) therapy succeeds in hematologic cancers, solid tumors such as TNBC remain challenging due to limited T-cell infiltration, an immunosuppressive tumor microenvironment (TME), antigen heterogeneity, and CAR-T-cell exhaustion. Macrophages naturally infiltrate TME and engulf pathogens, dying cells, and cancer cells. Engineering them with CARs creates CAR-Macrophages (CAR-Ms) that may infiltrate tissue more effectively, remove tumor cells by engulfment, activate broader adaptive immune responses, and reduce autoimmune risks. Early studies show CAR-Ms can convert the TME to a pro-inflammatory state, and initial clinical trials indicate safety and some anti-tumor activity, though complete tumor eradication is not yet established. We found that co-expression of active Rac2 (Rac2E62K) enhances CAR-M recognition and engulfment of living cancer cells, suggesting Rac-enhanced CAR-M (Race-CAR-M) could improve anti-tumor efficacy across cancers.

To test the anti-tumor activity of Rac2E62K macrophages in a more realistic 3D environment, we have adapted the chicken chorioallantoic membrane (CAM) assay to study tumor-macrophage interactions. This low-cost ethical model allows in vivo monitoring of angiogenesis and metastasis and can be used to test drug combination therapies with Race-CAR-Ms. Our ongoing work indicates that Rac2E62K macrophages cooperatively infiltrate the TNBC microenvironment and dramatically shrink tumors by

engulfing target cancer cells. Rac2E62K macrophages limit secretion of proangiogenic Vascular Endothelial Growth Factor (VEGF) and reduce neoangiogenesis. In addition, nanoparticle encapsulated PARP inhibitor (Olaparib) produces additive anti-tumor effects with Rac2E62K macrophages in the CAM tumor xenograft model of TNBC. We are currently engineering Race-CAR-Ms for TNBC by incorporating Rac2E62K together with CARs targeting TNBC specific tumor-associated antigens (TAA). Since Rac operates downstream of engulfment receptors in macrophages, our approach offers a tunable system for engineering CAR-M therapies against various cancers.

3. Targeted Therapeutics to Modulate Myeloid-Derived Innate Immune Cell Phenotype

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Small molecule immunotherapeutics are readily available as pharmacological interventions in inflammatory diseases. However, their efficacy is limited by poor solubility, rapid renal clearance, and non-specific cellular biodistribution. To address these challenges, our lab has previously developed an injectable polymer-nanoparticle (iPNP) hydrogel system with shear-thinning and self-healing properties for the minimally invasive, local delivery of immunomodulatory drugs. This hydrogel system is composed of cyclodextrin nanoparticle (CDNPs) drug reservoirs dynamically crosslinked by adamantane-modified hyaluronic acid (Ad-HA) via supramolecular guest-host interactions. Myeloid cells, including pro-inflammatory macrophages (MF) and their monocyte (Mo) precursors, drive pathological tissue remodeling post-myocardial infarction and eventual resulting ischemic heart failure (IHF). Modulating MF and Mo phenotypes to prevent IHF is a promising therapeutic approach. Preliminary drug screens revealed the small molecule celastrol as a potent inhibitor of inflammatory signaling and promoter of reparatory phenotypes in MF. However, drug effects on Mo remain to be examined. Here, we demonstrate the ability of celastrol to bias innate immune cells toward nonclassical, pro-reparatory phenotypes. A two-step drug screening process identified inhibitors of inflammatory markers in RAW-Blue MF reporter cells and identified drug candidates that promote reparatory phenotypes via transcriptional analysis. The chosen candidate, celastrol, demonstrated cell viability at doses up to 0.1 μM and was shown to bias primary Mo toward a nonclassical (CCR2-CX3CR1+) phenotype, even in a proinflammatory environment via flow cytometry. These results align with celastrol's demonstrated ability to bias MF toward a pro-reparatory phenotype. Consistently, monocyte migration toward bone marrow-derived macrophages (BMDMs) treated with celastrol was reduced. Further, celastrol significantly reduced expression of proteins related to NF-kB signaling and inflammatory Mo/MF recruitment in a 24-plex Luminex assay with human peripheral blood mononuclear cells (hPBMCs). These findings support the use of our locally-injectable iPNP hydrogel system for celastrol delivery post-MI, offering a versatile platform for immune modulation in ischemic injuries.

4. Microplastics induce tertiary lymphoid structure formation and autoantibody production within skeletal muscle after volumetric muscle loss

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Upon tissue damage, self-antigens that are otherwise obscure to immune cells are exposed. Consequently, chronic inflammation resulting from synthetic material implantation drives autoimmunity. By using a mouse model of volumetric muscle loss (wherein mice receive saline, polyethylene (PE) or decellularized extracellular matrix (ECM) at the site of muscle trauma), we sought to uncover mechanisms of pathogenesis and regulation of autoimmunity after tissue damage and biomaterial implantation.

Firstly, we observed that skeletal muscle damage in conjunction with microparticulate synthetic polymers like PE gives rise to tertiary lymphoid structures (TLS) in WT C57BL6/J mice. Within these ectopic germinal centers, we observed the formation of CD138+ Fas+ antibody secreting plasma cells. In contrast, plasma cell generation was significantly attenuated in mice which received ECM implants

and control injury. Counterintuitively, we found that CD8+ T cells are required for substantial plasma cell generation in TLS and dispensable in tissue draining lymph nodes. Similar analyses in NOD WT and NOD Aire-/- mice showed that synthetic microplastics (PE) synergize with Aire depletion to exacerbate plasma cell response in TLS.

Further, single cell and T cell receptor sequencing on muscle lymphocytes revealed a clonally diverse cluster of Ly49+ T cells which were enriched by ECM and depleted by PE. These cells shared an expression signature of Il2rb and Ikzf2 with Foxp3+ regulatory T cells and Xcl1, Ccl5 and Nkg7 with NK cells while uniquely expressing Klra6 and Ly6c2. Lastly, Ly49+ T cells expressed high levels of Fasl which encodes for the apoptotic molecule Fas-L. Correspondingly, B and T cells that formed TLS expressed Fas. Taken together, our data suggest that Ly49+ T cells regulate TLS."

5. Intramuscular mRNA delivery via steroid-containing lipid nanoparticles reduces LNP-induced inflammation and prevents autoimmune disease

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Autoimmune diseases affect 5-10% of the global population and result from the loss of central tolerance, leading to self-reactivity and inflammation. Current treatments focus on controlling inflammation but do not address the underlying loss of tolerance. Emerging therapies are exploring delivery of self-antigens—either as peptides or encoded via mRNA—to retrain the immune system and restore tolerance. However, intracellular delivery of these self-antigens is challenging due to several biological barriers. Lipid nanoparticles (LNPs) are well-known for their ability to deliver cargo intracellularly, as evidenced by the COVID-19 vaccines. Unfortunately, LNPs also induce acute inflammation or exacerbate pre-existing inflammation, limiting their applicability in autoimmune diseases. Here, we engineered LNPs with FDA-approved anti-inflammatory corticosteroids, aiming to deliver mRNA while mitigating inflammation. We screened 6 steroids in an SM102 LNP (Moderna) and found that incorporating triamcinolone (TRI) resulted in the best balance of effective mRNA delivery and reduction of NF-κB activity while maintaining LNP charactertistics. We then delivered our TRI LNPs intramuscularly in a mouse model of endotoxemia and found that TRI LNPs were able to significantly reduce levels of IL-1β, TNF, and IFNɣ compared to the levels induced by the control. Even with reduced inflammation, TRI LNPs maintained comparable mRNA delivery to the control, indicating that steroid incorporation improved the safety profile without compromising mRNA delivery. Finally, we tested the efficacy of TRI LNPs in a mouse model of multiple sclerosis (EAE). We found that intramuscularly administered TRI LNPs containing therapeutic mRNA were able to completely prevent paralysis compared to controls by inducing a tolerogenic immune response in the spinal cord. Specifically, we observed decreased T cell infiltration and antigen-specific Th17 cells, and increased antigen-specific Tregs in the spinal cord. Thus, we were able to demonstrate that intramuscular mRNA delivery via steroid-LNPs is able to restore tolerance and mitigate autoimmune disease.

6. How one virus infection awakens another silent virus by extracellular vesicles - Tale of two viruses and exosomes

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Individuals living with the chronic infection of Human T-cell Lymphotropic Virus type-1 (HTLV-1) is often associated with co-infections such as Epstein-Barr Virus (EBV) and associated lymphomas like Burkitt's lymphoma, Hodgkin lymphoma, and diffused large B-cell lymphoma (DLBCL). Prior studies, including our own, have shown that HTLV-1 infected cells release extracellular vesicles (EVs) that contain viral proteins, cytokines/chemokines, RNAs and even immune check point molecules like PD-1 etc. Small EVs such as exosomes are capable of modulating recipient cell function and facilitating viral spread. To delve deeper into the understanding of this mechanism, we exposed EBV-infected latent B cells with

HTLV-1-derived exosomes and observed a significant increase in multiple EBV genes related to viral replications (e.g. EBNA1, BMRF1, BZLF1, LPMs, etc.). In order to understand the mechanism of this observation, proteomic analysis was conducted followed by an extensive data analysis. Interestingly, proteins related to various relevant signalling pathways were differentially regulated such as Akt, mTOR, and NF-kB, which is critical for EBV replication (e.g. ACTB) Thus, we have expanded proteomic studies with a specific NF-kB inhibitor. Other co-signalling pathways such as Ras-MAPK pathway associated with cancer progression and metastasis (e.g. RASGRP3, CDK4/6) was identified that is involved in the regulation of oncoprotein MEF-2. Indeed, follow up in vitro studies have confirmed the induction of MEF-2 genes by HTLV exosomes in EBV positive B cells. These findings, together with activation of the NF-κB pathway, a known downstream target of HTLV-1 Tax and a regulator of inflammatory and survival responses, suggest a mechanism by which HTLV-1-derived exosomes may facilitate EBV reactivation and lymphomagenesis.

7. Lytic reactivation of EBV by HIV proteins and antiretroviral therapy with the heightened expression of MEF-2 oncogenes implicated in AIDS-related cancers

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Despite antiretroviral therapy (ART), people living with HIV (PLWH) remain at increased risk of developing AIDS-related cancers. This higher risk can be attributed to long-term ART use, exposure to soluble HIV proteins, and drugs of abuse. Epstein-Barr virus (EBV) is common in malignancies such as Burkitt's lymphoma and Hodgkin's lymphoma. Although ART is not curative, prolonged use can accelerate cancer progression. Herein, we investigated the effects of factors that can impact EBV latency in PLWH in cooperation with the oncoproteins, myocyte enhancer factor (MEF)-2 family. EBV negative (Ramos) and positive lymphoblastoid cell (LCL) lines were stimulated with Biktarvy (Tenofovir, Emtricitabine, and Bictegravir), recombinant HIV proteins (Nef and Rev), and/or drugs of abuse (cocaine, methamphetamine, and morphine). We observed that Nef, Rev, and ART promote expression of EBV lytic genes (e.g. BZLF1, BMRF) and oncogenes MEF-2A and -2C. MEF-2 observations were confirmed at the protein level and EBV gene changes were validated in HIV/EBV patient samples. Lytic reactivation of EBV was confirmed by DNA level changes, and is being assessed at the protein level of gene products, BZLF1 and BMRF1. Drugs of abuse alone did not show significant effects; however, with ART, Methamphetamine influenced viral gene expression and suppressed anti-inflammatory cytokines (IL-8 & IL-10) in a NF-KB-dependent manner, the key signaling pathway for EBV reactivation. Inhibition of NF-kB reversed the impact of HIV proteins and ART on EBV latent B cells. These findings establish a clear link between oncogenesis and persistent HIV infection, highlighting the oncogenic risks of HIV-associated factors.

8. Shear-thinning, injectable hydrogels for the automated and local delivery of cell therapies

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Cell therapies are a rapidly advancing field with the potential to transform personalized medicine and to address underlying biological dysfunctions rather than just managing symptoms. While effective in diffuse treatments (e.g., blood cancers), applications in solid tissue diseases (e.g., solid tumors, heart failure) remain limited by poor cell retention, survival, and a lack of reproducible delivery methods. To address these challenges, we developed an injectable, shear-thinning, and self-healing hydrogel as a cell-delivery vehicle, with the ability to enhance cell viability during syringe injection and localized retention post-delivery. Our platform uses hyaluronic acid (HA) modified with guest (adamantane, Ad) and host (β-cyclodextrin, CD) moieties. Upon mixing, these components self-assemble via reversible guest-host interactions into a dynamic polymer network. We characterized the material's rheological properties and assessed its cytocompatibility and protective effects in vitro. Rheological analysis

confirmed the hydrogels transition from solid-to-liquid at 100–150% strain, exhibit composition-dependent shear-thinning for injectability, and self-heal rapidly for post-injection cargo retention. In vitro, RAW264.7 macrophages, Jurkat cells, and adipose-derived stem cells remained highly viable (>75%) for over 48 hours following syringe injection, with optimal viability at lower polymer concentrations (2.5%w/v) and Ad modification (25%). In studies conducted in collaboration with Cellular Vehicles, Inc., cell viability (>95%) was maintained throughout fully-automated cell-laden hydrogel formulation using a herringbone microfluidic mixer and subsequent injection through a clinically-relevant 4 Fr catheter. In contrast, cell suspensions were <60% viable, demonstrating the hydrogel's protective capacity under high-shear conditions. This guest-host hydrogel system represents a promising platform for improving the targeted delivery and efficacy of cell-based therapeutics. By protecting cells during injection and localizing them at the target site, this strategy offers a significant advancement for treating solid tumors, promoting tissue regeneration, and enhancing therapeutic outcomes in translational medicine.

9. Development of Dendritic Cell Membrane-Coated Nanoparticles for Antigen-Specific T-Cell Engagement

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Cell membrane-coated nanoparticles represent a biomimetic nanotechnology platform that harnesses the inherent biological functions of source cell membranes, promoting receptor-specific interactions. Furthermore, these particles demonstrate the capacity to escape immune surveillance and prolonged systemic circulation. Owing to these advantages, membrane-coated nanoparticles have emerged as a delivery platform with excellent therapeutic efficacy across diverse biomedical applications. Dendritic cells (DCs) initiate adaptive immune responses by activating T cells during antigen presentation. This process is primarily driven by the cognate interaction between DC-derived pMHC (peptide-major histocompatibility complex) and the T-cell receptors (TCRs) and is further strengthened by costimulatory molecule engagement (CD80/86-CD28) and adhesive molecule binding (ICAM-1-LFA-1). DC membrane-coated nanoparticles (DCmPs) can recapitulate the cognate DC-T cell interaction and have shown significant therapeutic potential in vaccination and immunotherapy. However, our current understanding of the coating processes and the composition of the final products after coating is limited, significantly hindering the development of DCmP-based therapy. Here, using DC2.4 cells and poly (lactic-co-glycolic acid) (PLGA) nanoparticles, we report that a combined coating approach (sonication followed by extrusion process) achieved a high level of protein coating and exerted superior control over the diameter and uniformity of DCmPs relative to sonication or extrusion alone. Leveraging the homotypic interactions between DCmPs and DC2.4 cells, we determine that about 80% of PLGA cores are coated with membrane proteins, as confirmed by flow cytometry and confocal microscopy. Because DC2.4 cells predominantly express MHC class I (MHC-I) molecules, DCmPs show preferential binding to cognate B3Z CD8+ T cells rather than DOBW CD4+ T cells, confirming antigen-specific binding. Furthermore, we demonstrate that DCmPs activate B3Z CD8+ T cells in vitro, similar to DC2.4 cells. Collectively, these results establish DCmPs as a versatile platform with significant therapeutic promise for antigen-specific immunotherapy.

10. Mucins suppress neutrophil extracellular trap formation in human airways

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During infection and in chronic lung diseases such as cystic fibrosis, chronic obstructive pulmonary disease, or asthma, there is often neutrophilic inflammation which when left uncontrolled can lead to severe lung damage. Neutrophils influx into the airways and release neutrophil extracellular traps (NETs) within the airway mucus in an inflammatory response known as NETosis. During NETosis, nuclear chromatin is decondensed and cellular membranes disintegrate to produce a web-like structure composed of DNA, histones, and various granular proteins. However, it is unknown how the airway

mucus itself modulates NETosis. Mucins are large, heavily glycosylated biopolymers that are the primary structural component of the mucus gel. There are two main secreted mucins that form the mucus gel in the airways, mucin 5B (MUC5B) and mucin 5AC (MUC5AC). Previous research has demonstrated that cervical mucins suppress NETosis, while salivary mucins induce NETosis. Both the cervical and salivary mucins were shown to modulate NETosis via glycan-dependent interactions. Given the role of neutrophil-driven immune dysfunction in chronic lung disease, we investigated the effects of human airway mucus and the collective function of each airway mucin subtype on NETosis. Specifically, we evaluated the effects of mucus derived from normal human airway epithelial (HAE) cells and HAE cells genetically engineered using CRISPR/Cas9 to knock out expression of the secreted mucin proteins MUC5B or MUC5AC on NETosis. We found that normal airway mucus is highly modulatory of NETosis, and that both airway mucin proteins contribute to dampening NET release. Further, we found using a co-culture model of neutrophils and differentiated HAE cultures that NETosis occurred more readily in cultures deficient in either MUC5B or MUC5AC. Finally, to translate our findings into novel immunomodulatory therapies, we are engineering mucin-coated nanoparticles for the prevention of NETosis in chronic lung diseases.

11. Metabolomic Analysis of Mycobacterium Abscessus Infected Macrophages

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Spatial metabolomics, which allows in situ detection and high-dimensional analysis of low-molecular-weight metabolites (< 1500 Da) has been utilized to identify metabolic markers/patterns relevant to antimicrobial resistance. Metabolomic studies have been conducted on various mycobacteria, however, Mycobacterium abscessus (Mab) remains relatively unexplored. Mab is a rapidly growing, multi drug-resistant nontuberculous mycobacterium (NTM) that causes severe infections in patients with underlying lung disease and those who are immunocompromised. Therapy is prolonged, consisting of both oral and intravenous antibiotics including the beta-lactam, imipenem (IPM). Due to Mab's intrinsic resistance to many antibiotics, alternative, clinically validated, treatment options are being explored including immunomodulators such as interferon gamma (IFNg), a type II interferon. We aim to characterize the metabolomic profile of Mab infected THP1 macrophages during treatment with IFNg and IPM alone or in combination to better understand the host cell-bacteria microenvironment. By identifying metabolic pathways involved both host cell and pathogen, we hope to elucidate bacterial virulence and cellular mechanisms of infection control. After cells were infected with mCherry-Mab and treated for 24 hours, spatial MALDI-TOF of the fluorescent areas was performed to detect all metabolites produced. Data analysis was carried out to annotate the metabolites and pathway analyses were run to highlight any discriminatory metabolites, leading to the identification of affected pathways. Comparison of conditions permitting bacterial clearance versus persistence will identify metabolic changes occurring during successful Mab treatment and provide the basis for additional studies aimed at maximizing those changes to improve anti-Mab therapy. This research was supported by the Intramural Research Program of the National Institutes of Health (NIH). The contributions of the NIH author(s) were made as part of their official duties as NIH federal employees, are in compliance with agency policy requirements, and are considered Works of the United States Government. However, the findings and conclusions presented in this paper are those of the author(s) and do not necessarily reflect the views of the NIH or the U.S. Department of Health and Human Services.

12. IL-12 mRNA lipid nanoparticles to treat metastatic ovarian cancer

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Ovarian cancer is the most lethal gynecologic malignancy, as 70% of patients are diagnosed once their disease has distantly metastasized throughout the intraperitoneal (IP) cavity as small tumor spheroids. This spread limits the efficacy of traditional frontline therapies, such as cytoreductive surgery and intravenous (IV) chemotherapies. In recent years, cytokine-based immunotherapies have shown clinical

promise in enhancing anti-tumor immunity, however effective and safe delivery remains challenging as current recombinant protein-based approaches have short half-lives and can trigger a toxic “cytokine storm” when administered intravenously (IV). To overcome this barrier, we investigated the intraperitoneal (IP) delivery of interleukin-12 (IL-12) mRNA to metastatic ovarian cancer via ionizable lipid nanoparticles (LNPs), the most clinically advanced, non-viral platform for nucleic acid delivery.

We first identified an LNP formulation that significantly improved the transfection of luciferase mRNA within ovarian cancer cells in vitro. Comparing this LNP’s biodistribution within a syngeneic model of metastatic ovarian cancer, IP delivery of LNPs conferred ~1,000-fold greater mRNA transfection within tumors compared to IV administration. However, given significant mRNA delivery to peritoneal organs, we chose IL-12 as our therapeutic cargo, aiming to stimulate and reconstruct the immunosuppressive tumor microenvironment that is characteristic of metastatic ovarian cancer. Mice treated IP with IL-12 mRNA LNPs demonstrated significant and lasting tumor regression compared to IV IL-12 mRNA LNPs, IP IL-12 protein, IV IL-12 protein, and PBS controls. IP IL-12 mRNA LNPs mediated increases in ascites-derived CD4+ effector memory and CD4+ and CD8+ central memory T cells and decreases in CD4+ regulatory T cells, as well as increases in splenic CD4+ memory and CD8+ central memory T cells. Additionally, serum-based assays demonstrated no adverse effects of IP IL-12 mRNA treatment on hepatic and pancreatic enzymes. These findings underscore the potential of LNPs as a clinically relevant platform for mRNA-based immunotherapy in metastatic ovarian cancer.

13. Optimal Extracellular Viscoelasticity Tunes Cytoskeletal Dynamics to Maximize T Cell Activation and Epigenetic Remodeling
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At the immunological synapse, actomyosin reorganization and retrograde flows are regulated by the mechanical properties of the antigen presenting cell (APC), enabling T cell spreading and effector activation. Although APCs exhibit viscoelastic behavior, the extent to which their relaxation dynamics, interacting with T cell receptors of varying mechanical strengths, reshape cytoskeletal organization and govern force transduction for mechanosensing remains unexplored. Our findings on T cell mechanosensing suggest that activation is maximized on substrates with intermediate viscoelasticity, where cellular and substrate timescales are optimally matched. Using both computational modeling and Jurkat T cell experiments, we found that spreading area, traction forces, and ZAP70 phosphorylation all peak at an intermediate viscosity, reflecting enhanced mechanical coupling and prolonged bond lifetimes at the immune synapse. This regime supports effective force transmission and sustained receptor ligand engagement, both essential for downstream signaling. Incorporating kinetic proofreading into our motor clutch model, we show that optimal substrate viscosity prolongs bond engagement just enough to enable productive phosphorylation events. Furthermore, integrins such as LFA-1 stabilize synaptic mechanics by reducing retrograde flow and extending T cell receptor (TCR) bond lifetimes. We experimentally validated Jurkat T cell responses across a range of substrate stiffness and viscoelasticity, observing a biphasic pattern in both spreading area and nuclear size, with maxima at intermediate stiffness and viscosity. Global DNA acetylation displayed a similar biphasic response, suggesting maximal chromatin decondensation at these intermediate viscoelastic conditions. Collectively, our results indicate that adjusting extracellular matrix viscoelasticity can epigenetically shape T cell fate, offering strategies to enhance CAR T cell therapy through mechanical optimization of engineered environments.

14. Targeting CD87 in Colorectal Cancer: A High-Throughput Strategy for CAR-T Cell Design
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Colorectal cancer (CRC) is predicted to be responsible for nearly 53,000 deaths in the U.S. in 2025, with around 150,000 new cases diagnosed. These numbers emphasize the urgent need for innovative therapies for CRC. Chimeric Antigen Receptor T-Cell Therapy (CAR-T) is already employed in the

treatment of some blood cancers. One of the obstacles that CAR-T faces in its application to solid tumors is the limited tumor antigen specificity of this therapy and the unacceptable on-target/off-tumor toxicity. CD87, the receptor for the urokinase plasminogen activator (uPAR), is markedly overexpressed in CRC and shows low transcript abundance in most healthy tissues, suggesting that it might be an attractive target antigen.

Methods: To assess the potential safety of targeting CD87, we analyzed publicly available bulk and single-cell RNA sequencing (scRNAseq) datasets from healthy human and mouse tissues. Next, we engineered CD87-directed CAR-T cells (CART87) and assessed their cytotoxicity against CD87-positive CRC cell lines (LS174T, SW480, T84) compared with CD87-negative HEK293 cells. To further optimize CAR design, we employed a Jurkat NFAT-Lucia reporter assay, which quantitatively measured CAR activation upon stimulation with recombinant uPAR and CD87-expressing cells. This system enabled rapid comparison of single-chain variable fragments (scFv) and structural domains configurations.

Results: At the tissue and organ level, HPA reports that CD87 mRNA expression is highest in the bone marrow, followed by urinary bladder, appendix, gallbladder, and lung. Single-cell data of healthy bone marrow, whole blood leukocytes, lung, and urinary bladder revealed that CD87 mRNA expression is largely restricted to mature monocyte-lineage cells. Murine organs/tissues produced similar results. In vitro, CART87 demonstrated strong, specific killing of all tested CD87-positive colorectal cancer cell lines while sparing the CD87-negative control. Reporter-based screening identified a lead scFv and CAR design with robust CD87-specific activation.

Conclusions: This work identifies CD87 as a promising, selectively expressed target antigen for development of CAR-T therapy in colorectal cancer, supporting the advancement of CART87 toward preclinical and in vivo validation. In addition, we describe the rational design of a CART87 construct using a high-throughput strategy that could be broadly applied in CAR design for other target antigens.

15. Prior targeted therapy treatment modulates the effects of Axl inhibition on myeloid cells in the human in vitro melanoma tumor microenvironment

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Immune checkpoint inhibitors have revolutionized cancer therapy; however, their success is limited to patients with prior T cell infiltration. To increase the breadth of patients benefitting from this therapeutic modality, immunologically cold tumors need to be converted to hot ones rich with T cells. Axl, a member of the Tyro3, Axl, and MerTK receptor tyrosine kinase family, is an attractive target for this purpose. Axl inhibition is not only known to promote tumor cell death and inhibit tumor cell invasion and migration, but also thought to promote T cell infiltration into the tumor. While pre-clinical studies in mice have demonstrated the potential of Axl inhibition to have dual – tumoricidal and immunostimulatory – therapeutic effects, clinical trials have reported limited efficacy. This discrepancy in translation may be explained in part by an incomplete understanding of the immunological effects of Axl inhibition, specifically on the Axl-expressing myeloid compartment of the tumor microenvironment (TME). Herein, we developed and characterized a human in vitro model system to investigate the effects of Axl inhibition on myeloid cells in the context of the melanoma TME. Bemcentinib is an Axl-specific small molecule inhibitor in clinical trials for a variety of cancer indications. Bemcentinib is known to promote myeloid cell activation, which we observed in co-cultures of macrophages and tumor cells. Upon the addition of dendritic cells to pre-existing macrophage and tumor cell co-cultures, the magnitude of the macrophage response was dampened, suggesting crosstalk and coordination between these myeloid cell types. Notably, we found that treatment-naive and targeted therapy-treated tumor cells had distinct effects on myeloid activity, which affected their responses to bemcentinib treatment. We are actively investigating changes in cell signaling that underlie these observations. Overall, our work demonstrates the importance of studying Axl inhibition in the context of a tumor immune landscape defined by standard-of-care treatments.

16. Targeting the Adenosine–ADA-1 Axis to Restore HIV-Specific CD8⁺ T Cell Function

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Achieving a functional HIV cure remains a global priority. A major barrier is the loss of HIV-specific CD8⁺ T cell function, driven by the chronicity of HIV infection. Current strategies to reverse CD8⁺ T cell dysfunction show promise in cancer but have limited efficacy in people living with HIV (PLWH), underscoring the need for approaches tailored to this unique immunologic landscape. We propose targeting the adenosine (ADO) signaling pathway—an inflammation regulator that is dysregulated in HIV.

The balance between inflammatory ATP and immunosuppressive ADO serves as a key local signal distinguishing immune activation from suppression. Under normal conditions, ADO levels are tightly controlled by adenosine deaminase-1 (ADA-1), which irreversibly converts ADO to inosine, preventing excessive immunosuppression while also enhancing immune activation. In PLWH, this ADO/ADA-1 axis is dysregulated with increased expression of ADO-generating enzymes and reduced ADA-1 expression—driving suppressed immune activation and contributing to viral persistence.

We hypothesize that ADO signaling disproportionately impairs HIV-specific CD8⁺ T cell function and that enhancing ADA-1 expression can reverse this suppression. Using an ex vivo model, we show that CD8⁺ T cells from PLWH are highly susceptible to ADO-mediated suppression in an ADA-1–dependent manner. To overcome this, we developed CD8-targeted lipid nanoparticles (CD8-LNPs) to deliver ADA-1 mRNA, achieving selective overexpression in primary CD8⁺ T cells. This approach leverages ADA-1's dual role in degrading excess extracellular ADO and enhancing immune function by promoting T cell activation and dendritic cell maturation. Together, these findings provide a foundation for ADA-1 as a novel and direct means to restore HIV-specific CD8⁺ T cell function, highlighting the potential for ADA-1–based immunotherapy to boost antiviral immunity and advance toward a functional HIV cure.

17. Development of Avidity-Controlled Biotherapeutic Delivery Systems for the Treatment of Acute Kidney Injury

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Acute kidney injury (AKI) occurs in over half of critically ill patients and nearly one-third of critically ill children. Independent of cause, AKI is characterized by a hyperinflammatory milieu that drives the development of chronic kidney disease (CKD), which affects 37 million Americans. Regulatory T cell (Treg) dysfunction, including their poor recruitment and differentiation in AKI to mitigate inflammation, is associated with chronic kidney disease (CKD) development. Here, we develop injectable, granular 'host'-hydrogels as an affinity-based drug delivery system to sustain local chemokine and cytokine delivery. We aim to prevent AKI-to-CKD progression by i) attracting CD4+ T cells to the site via rapid chemokine release and ii) sustaining low-dose 'guest'-modified IL-2 delivery to induce Treg differentiation. Methacrylated β-cyclodextrin and dextran (MeCD, DexMA) were co-polymerized (5mM LAP, 10mW/cm2) to yield shear-thinning, injectable hydrogels (G'~15kPa) with a high host concentration. Biocompatibility was assessed in healthy BalbC mice (15µL kidney subcapsular injection) relative to saline injection controls; kidney function was assessed (transdermal glomerular filtration rate, blood urea nitrogen, and serum creatinine) over 28 days. Migration assays comparing established chemokines identified CCL21 as a potent chemokine for activated CD4+ T cells. Supramolecular guests (adamantane, Ad) were conjugated to IL-2 via NHS catalysis (5 Ad per IL-2) with only moderate loss of bioactivity. Hydrogels (50µL) loaded with CCL21 and/or Ad-IL2 were injected subq in healthy mice, with T cells quantified by flow cytometry. Post-injection, CCL21 (25µg) recruited CD4+ T cells to the hydrogel site within the first week, while co-delivery with Ad-IL2 (5µg) increased Treg populations over 28 days without promoting cytotoxic CD8+ T cell responses. Overall, we

identified CCL21 as a potent chemokine to attract T cells, which in combination with bioactive Ad-IL2 increases local Treg abundance. Such controlled release is a promising approach to modulate the immune microenvironment post-AKI, preventing CKD development.

18. Resolving Type 2-Polarized T Follicular Helper Cells in Human Peripheral Blood
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CD4 T cells are an essential component of the immune system that coordinate protective responses during vaccination, infection, and other disease contexts. CD4 T cells can differentiate into subsets such as T Helper (Th) and T Follicular Helper (Tfh) with distinct functions. Th cells are grouped into Th1, Th2, and Th17 subsets based on expression of surface proteins and transcription factors. Compared to Th cells, Tfh more potently stimulates antibody production by B cells. However, recent studies demonstrate Tfh can ‘polarize’ toward Th1, Th2, and Th17, yielding Tfh1, Tfh2, and Tfh17 subsets. Tfh1 and Tfh17 surface proteins in humans are well characterized, but Tfh2 cells remain poorly defined. There is no standard set of surface markers to identify Tfh2, and their expression of common Th2 markers remains unclear. We therefore sought to evaluate whether Th2 markers such as CCR3, CCR4, CCR8, or CRTH2 are expressed in Tfh2 cells and how they correlate with GATA3, a transcription factor known to drive Th2 differentiation. By analyzing Th2 surface markers and GATA3 expression in human CD4 T cell populations using spectral flow cytometry, our goal is to better define Tfh2 identity. Defining the Tfh2 subset is critical, as targeting subsets like Tfh2 through vaccination may lead to stronger, longer-lasting protection against pathogens. Vaccine responses are one of the most effective ways we prevent infectious disease and protect vulnerable communities.

19. CCL5/CCR5 axis inhibition as a strategy to prevent drug resistance in BRAF-mutant melanoma
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In cutaneous malignant melanoma, treatment is often complicated by the development of resistance to both first-line immunotherapies and second-line targeted therapies. Patients with BRAFV600E mutations typically show reduced responses to immunotherapy in the treatment-naïve setting, which are further diminished following prior targeted therapy exposure. In BRAFV600E-mutant melanoma pre-clinical models, treatment with second-line BRAF and MEK inhibitors (BRAFi+MEKi) drives CCL5 production by tumor cells, leading to the recruitment of myeloid-derived suppressor cells (MDSCs) to the tumor microenvironment. MDSCs are potent immunosuppressive cells that secrete inhibitory molecules (such as IL-10, TGF-β, and ARG-1) and other suppressive factors (including reactive oxygen species [ROS] and nitric oxide [NO]), thereby promoting a shift from a pro-inflammatory "hot" immune state to an immunosuppressive "cold" state. This immune transition facilitates tumor immune evasion and serves as a mechanism of drug resistance. Our findings suggest that disrupting the CCL5/CCR5 signaling axis—via novel drug-loaded nanoparticles—could limit MDSC recruitment, restore a favorable immune microenvironment, and enhance anti-tumor immunity and therapeutic efficacy. Our results suggest that blocking the undesired immune-suppressive effects of targeted therapy may overcome drug resistance in melanoma, including in settings where sequential therapy approaches are clinically necessary.

20. Local delivery of amphiregulin from oxidized alginate hydrogels enhances regeneration during ischemic injury
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Ischemia, the loss of blood flow as a result of injury or disease, affects millions annually. Loss of blood flow in peripheral limbs leads to muscle degeneration, denervation, and tissue necrosis. Regulatory T-cells (Tregs) are anti-inflammatory immune cells that suppress pro-inflammatory responses and secrete

factors that drive muscle stem cell differentiation and innervation. However, Tregs are recruited to severe injuries at low frequencies, resulting in excessive inflammation and fibrosis. Amphiregulin (AREG) is a potent cytokine that activates Tregs, muscle satellite cells and motor neurons. We engineered an injectable, oxidized (degradable by hydrolysis), calcium cross-linked alginate hydrogel for local and sustained release of AREG at ischemic injury sites. Hydrogels were formulated using very low and medium viscosity alginate (75:25 ratio), oxidized at varying percentages, loaded with 2 µg AREG, and crosslinked with calcium sulfate. AREG incorporation into alginate hydrogels resulted in moderate burst release followed by sustained release over 14 days. Increased alginate oxidation enhanced total AREG release. 50 µL of hydrogel with or without AREG was injected intramuscularly into the limbs of female BALB/c mice 24 hours after undergoing femoral artery, vein, and nerve ligation. While AREG delivery did not significantly improve blood perfusion return, it reduced limb necrosis progression and severity compared to blank hydrogel controls. By day 14, AREG-treated mice exhibited enhanced walking gait patterns compared to minimal uncoordinated movement in blank hydrogel-treated mice. Qualitative histology analysis suggests that AREG hydrogel reduces fibrosis and enhances muscle regeneration in ischemic muscle compared to blank hydrogel controls. Overall, these findings suggest that sustained delivery of AREG from our hydrogel potentiates AREG's ability to drive tissue regeneration and reduce necrosis in ischemic injuries. Our ongoing work will quantify histological and functional innervated muscle repair, as well as measure specific immune cell recruitment in response to AREG delivery.

21. Engineering a responsive biomaterial for localized, inflammation-triggered delivery of anti-inflammatory drugs

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Osteoarthritis (OA) is a progressive musculoskeletal disease primarily characterized by inflammation-induced degeneration of the cartilage matrix, ultimately resulting in diarthrodial joint failure. Mesenchymal stromal cells (MSC) are promising candidates for cartilage regeneration in OA due to their multipotent differentiation potential and capacity to produce cartilage-specific matrix. However, the chronic inflammatory microenvironment in OA impairs MSC differentiation and matrix deposition, hindering therapeutic efforts. Corticosteroids such as dexamethasone (Dex) can reduce OA inflammation and pain, but long-term use leads to severe systemic side effects, undesired cartilage calcification, and chondrocyte apoptosis. Moreover, Dex is rapidly cleared from the joint with an intra-articular half-life of less than 12 hours, underscoring the need for localized, inflammation-triggered delivery systems that minimize off-target effects. To address this, our objective is to engineer an inflammation-responsive biomaterial capable of releasing Dex only in inflamed environments, thereby preserving MSC chondrogenic capacity for OA cartilage repair. To achieve this, Dex was tethered to an inflammation-sensitive peptide (Dex-pep) using solid-phase peptide synthesis and purified via reversed-phase high-performance liquid chromatography. Successful synthesis was confirmed using matrix-assisted laser desorption/ionization time-of-flight and proton nuclear magnetic resonance spectroscopy. Bioactivity testing showed that 300 nM Dex-pep reduced caspase-3/7 activity in MSCs exposed to inflammatory conditions, supported MSC chondrogenesis comparable to standard Dex treatment, and decreased both intra- and extracellular inflammatory macrophage markers. We evaluated drug release kinetics under inflammatory conditions with a fluorescent model using Cyanine-3 tethered to the inflammation-sensitive peptide (Cy3-pep) and conjugated to polycaprolactone (PCL). Cy3-pep-PCL scaffolds were 3D printed and incubated in inflammatory media. Fluorophore release studies demonstrated sustained release over one week under inflammatory conditions while Cy3 physiadsorbed onto PCL scaffolds was rapidly released. These results demonstrated a bioadaptive, inflammation-responsive platform that can suppress OA-associated inflammation locally while supporting MSC-mediated cartilage repair.

22. Engineering IL-12 Therapies for Ovarian Cancer

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High-grade serous ovarian cancer (HGSOC) is the deadliest gynecological cancer, with about 80% of cases diagnosed at stage III or IV. The current standard of care, which involves debulking surgery and platinum-based chemotherapy, achieves an initial reduction in tumor burden; however, most patients relapse, leading to a 5-year survival rate of roughly 30%. While recent advances in immunotherapies, particularly immune checkpoint inhibitors (CPI), have shown success in various cancers, they have been ineffective in HGSOC, likely due to low lymphocyte activity within the tumor microenvironment. Recent studies have shown promise for immune activation to stimulate the tumor microenvironment (TME) with activated lymphocytes using cytokines like interleukin 12 (IL-12) holds promise; however, systemic delivery of IL-12 has caused fatalities in clinical trials. In this study, layered lipid nanoparticles (LLNPs) are used to deliver mRNA encoding a targeted cytokine construct, enhancing IL-12 effects and offering a promising curative approach for HGSOC. We demonstrate that coating LNPs with polyanionic layers, such as poly-L-glutamine (PLE), increases transfection efficiency in vitro and improves affinity for cancer cells compared to unlayered LNPs. In vivo, layering reduces IL-12 immunotherapy toxicity, resulting in safer treatment for B6C3F1 mice bearing HM-1 tumors. The success of IL-12 therapies is enhanced by using targeted protein constructs for anchoring IL-12, like the CD45-anchored aCD45nb-IL12 mRNA cargo. This anchored cytokine construct has led to a curative therapy with long-lasting effects, eliminating the need for secondary treatments like CPIs. Additionally, various dosing strategies are tested to further improve the efficacy and safety of IL-12 therapy, aiming to minimize cytokine-related toxicity. The optimized PLE LLNP IL-12 therapy enhances immune cell infiltration into the TME, improves cytokine transport to tumor-draining lymph nodes, and reduces toxicities compared to unlayered LNPs, offering a promising curative approach for HGSOC.

23. Targeted immunotherapy to prevent myocardial infarction induced heart failure

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Myocardial infarction (MI) is a leading cause of heart failure (HF), contributing to 695,000 deaths annually in the U.S [1,2]. Despite advances in medical treatments, effective therapies to prevent HF following MI remain limited. One often overlooked driver of post-MI HF is prolonged inflammation directed by macrophages. Anti-inflammatory cytokines can polarize macrophages from an inflammatory to a reparative phenotype, reduce infarct size, and improve heart function, but their clinical application remains challenging [5]. Cytokines alone often achieve only transient and low-level activity in target tissues despite reasonable systemic doses due to poor tissue retention and rapid serum clearance. Furthermore, cytokines can have unwanted off-target toxicities in healthy tissues with frequent or excessive dosing [6]. To address this, we engineered a nanobody-based immunocytokine which targets the infarcted heart tissue and polarizes macrophages to a reparative phenotype. To determine therapeutic efficacy, we permanently ligated the left anterior descending coronary artery to model MI in mice. We found that our immunocytokine, administered in the first the week after MI, significantly improves cardiac contractility measured 4 weeks later. This benefit was attributed to the reduction in fibrosis and increased polarization of reparative macrophages in the infarct that we observed only with treatment using an infarct-targeted immunocytokine and not with a size-matched untargeted cytokine control. Importantly, our targeted immunocytokine shows minimal off-target effects in healthy tissues. These results highlight how a timely and localized treatment is necessary to effectively and safely prevent the excessive inflammation that catalyzes HF. These findings present a first-in-kind infarct-targeted immunocytokine therapy to improve healing and prevent HF after MI with minimal systemic side effects.

[1] Tsao, C.W., et al. Circulation. 2023. [2] Mensah, G.A., Roth, G.A. & Fuster, V. J Am Coll Cardiol. 2019 [3] Nahrendorf, M., et al. J Exp Med. 2007. [4] Kim, Y., et al. Int J Mol Sci. 2021 [5] Shintani, Y., et al. Sci Rep. 2017. [6] Momin, N. Curr Opin Biotechnol. 2023."

24. Implantable Bioinstructive Scaffolds for In Vivo CAR T Cell Manufacturing and Delivery

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Chimeric antigen receptor (CAR) T cell therapies have revolutionized the treatment of hematologic cancers and are a promising immunotherapy for treating solid tumors. However, they are often limited by the immunosuppressive tumor microenvironment due to poor CAR T cell persistence and tumor infiltration contributed by the expensive, lengthy, and labor-intensive ex vivo manufacturing process. These challenges highlight the urgent need for cost-effective and innovative strategies to enhance CAR T cell therapy for hematologic and solid tumors.

The Brudno Lab has developed subcutaneously implantable scaffolds that enable in vivo CAR T cell transduction, expansion, and release, serving as an all-in-one manufacturing and delivery platform. “MASTER” presents appropriate signals for T cell activation and expansion in addition to its static transduction properties. Our approach enhances CAR T cell efficacy in treating hematological cancers, outperforming conventional CAR T therapy.

Despite these advantages, the in vivo CAR T cell production and release kinetics within MASTER remain undetermined. We will image each component of interest—antibodies, cytokines, CAR T cells, and tumor cells—over time to gain mechanistic insights into the system's dynamics. Additionally, MASTER's manufacturing process can exhibit batch-to-batch inconsistencies. Therefore, we propose two derivative scaffold configurations that are easier to manufacture, though their impact on CAR T cell production, efficacy, and safety requires evaluation for translational applications.

25. Harnessing Tangential Flow Filtration and Hydrogels to enhance Transduction and Select Distinct Populations for CAR T Manufacturing

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Autologous chimeric antigen receptor (CAR) T-cell therapies have become an important tool for the treatment of hematological cancers. Despite this, CAR T has faced limitations in usage clinically, owing to challenges in their manufacturing process resulting in high costs and variable product quality. Current manufacturing results in inefficient and costly use of viral vectors, with minimal control over viral integrations per cell—causing heterogeneous CAR expression and variable therapeutic outcomes. Moreover, existing methods lack precise control of the CD4/CD8 T-cell ratio, a critical determinant of overall treatment efficacy. In this work, we use tangential flow filtration (TFF) with a ligand functionalized hydrogel coated membrane (HCM) to evaluate the impacts of cell density and flow patterns for enhancing and optimizing transduction and selection.

To demonstrate the value of our platform for lentiviral transduction, we transduced Jurkats with a model lentivirus. Modulation of cell density enhanced transduction: 5x and 1.5x in the device in comparison to static and spinfection, respectively, at low multiplicities of infection. Furthermore, TFF transduction reduced the mean fluorescence intensity of transduced cells, indicating that the TFF enhances transduction while minimizing vector integrations per cell. Additionally, the scalability of this platform was examined, demonstrating the ability to decouple operating parameters for optimal transduction.

To evaluate selection with our platform, we tested enriching CD8 T-cells from a heterogeneously mixed population of primary human pan T-cells and transducing the selected population in-situ with CAR lentivirus. In the selected population CD8+ T-cells were enriched ~20-30% and the %CAR+ cells were

enriched ~2x. Killing efficacy of these cells were then assessed and compared to static and non-enriched controls.

In summary, the use of TFF/HCMs enables more efficient use of lentiviral vectors and better control of cell therapy CD4/CD8 ratios, offering the potential to lower costs, increase accessibility, and increase efficacy of the therapy ."

26. Dimerization-mediated Synthetic Receptor Activation of Intracellular Signaling
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Engineered receptors typically rely on transcriptional reprogramming or proteolytic cascades, which can be suboptimal for certain immune cell types and often bypass native signaling mechanisms. We present a modular receptor platform that activates canonical immune pathways through ligand-dependent receptor dimerization. These synthetic receptors are agnostic to the extracellular ligand-binding domain, enabling flexible recognition of diverse soluble factors while maintaining the intracellular architecture required for integration with endogenous signaling. Our results show that coupling ligand-induced dimerization with designed receptor geometry amplifies pathway activation through transient interactions, producing robust and tunable signaling outputs. In multiple model cell systems, the receptors rewire cellular responses to soluble cues, eliciting native downstream programs with high specificity and fidelity. This approach provides a broadly applicable strategy for controlling immune cell response to soluble environmental signals, with potential utility in cell-based immunotherapies.

27. Jogging the Immune System: Mechanical stimulation using Low Intensity Vibration reduces T cell exhaustion
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Exercise imparts wide-ranging benefits on the body, from enhancing bone and muscle density to slowing the effects of aging. While often attributed to metabolic effects, exercise may also act directly through mechanical stimulation of tissues. Low-Intensity Vibration (LIV), which replicates the mechanical components of exercise, has been shown to reduce adipogenesis in mesenchymal stem cells in vitro and tumor burden in murine multiple myeloma in vivo. Recent findings show that LIV-treated T cells increase expression of activation receptors CD25 and CD69, as well as production of the pro-inflammatory cytokines IL-2 and TNFα, indicating that LIV directly stimulates T cell activation and may increase the adaptive immune response. However, many tumors evade immune detection by driving T-cell exhaustion via activation of inhibitory immune checkpoints. One such checkpoint, PD-1, is a critical target for immune evasion. Here, we evaluated if LIV influences PD-1 expression in exhausted T cells. To test this, primary T cells were cultured in RPMI 1640, 10% FBS, 1% Pen-Strep, and chronically activated using anti-CD3/CD28 Dynabeads (1:10 bead-to-cell ratio, replaced every passage on M/W/F) to induce exhaustion. Exhausted cells were then treated with five days of LIV (30 Hz, 0.35g, two 1hr daily bouts). Flow cytometry revealed that LIV-treated cells exhibited a 19% reduction in PD-1 expression relative to non-vibrated controls (n=11, p<0.01). LIV's potential to decrease PD-1 expression may make LIV-treated cells less sensitive to tumor inhibitory signaling, possibly improving their anti-cancer response. More work is needed to assess the immunomodulative capabilities of LIV on both T cells and tumor cells, and determine if LIV could directly augment T-cell cytotoxicity. Nonetheless, LIV's potential to mechanically modulate T cell sensitivity makes it attractive as a minimally invasive, non-drug adjunct to existing cancer immunotherapies.

28. Liver-detargeted aromatic bioreducible mRNA lipid nanoparticles confer lymph node tropism and robust antigen-specific immunity
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Lipid nanoparticles (LNPs) have been immensely successful in facilitating nucleic acid delivery to tissues of interest and are continuing to be optimized for mRNA delivery. These vehicles are particularly advantageous for vaccination due to their ease of production, highly tunable composition, and ability to induce a strong immune response, particularly without an additional adjuvant, as demonstrated most recently by the clinical success of the U.S. Food and Drug Administration (FDA)-approved Pfizer/BioNTech and Moderna COVID-19 mRNA vaccines. However, LNPs are known to exhibit strong liver tropism, which can cause hepatic toxicity and autoimmune responses for some individuals. To address this limitation, we developed a library of aromatic bioreducible ionizable lipids that potently transfect secondary lymphoid tissues with liver detargeting capabilities compared to the current gold standard ionizable lipid used in the COVID-19 vaccine. The lipids in this library consist of three modular components: amine core structure, lipid tail length, and regiochemistry. Our novel aromatic ionizable lipids employ benzene rings both as a scaffold for regiochemical differences and as a moiety to improve transfection. Bioreducible disulfide bonds in the lipids additionally serve to increase their biodegradability. When these aromatic ionizable lipids are formulated as LNPs, we demonstrate that top performing aromatic LNPs (aroLNPs) successfully accumulate in and transfect immune organs while evading hepatic tropism. We further demonstrate that top performing aroLNPs induce a strong antigen-specific immune response in mice when utilized in a preclinical SARS-CoV-2 vaccine study. Thus, aroLNPs represent an exciting platform for use in vaccines and other immune-related applications.

29. Investigating T cell pathophysiology in bone marrow of tibia fracture model of pain

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Complex regional pain syndrome (CRPS) is a debilitating pain condition that often manifests after a trauma. Current treatment options for CRPS are limited due to its complicated pathology and symptoms. It is increasingly evident that immune and autoimmune mediators in CRPS contribute to the development of pain, but the exact mechanisms are still unclear. Our previous studies showed alterations in miRNA profiles of individuals with CRPS, including a subset that showed a reversal in expression in patients that responded to ketamine treatment. One of these miRNAs, miR-25-3p, is predicted to target T cell activation genes implicated in autoimmune disease. Additionally, we observed an altered T cell phenotype in bone marrow from a rodent tibia fracture model (TFM) of CRPS, potentially representing a new pathological population that could be therapeutically targetable. We hypothesize that 1) miR-25-3p is an important regulator of T cell activation and 2) T cells in bone marrow are an active pathological population in CRPS. Our preliminary data demonstrates a reduction of miR-25 expression during activation and polarization towards Th17 and Treg phenotypes. We are currently testing if overexpression of miR-25 mimic stunts activation response and phenotypic expansion of naïve T cells, and if inhibiting miR-25 promotes activation and expansion. These studies will determine if miR-25 expression has direct effects on T cell function and polarization. Studies are also ongoing to investigate T cell phenotype and miR-25 expression in bone marrow from TFM mice by utilizing fluorescence-activated cell sorting and ex vivo polarization assays to determine if there is a link between T cell phenotype and pain. Collectively, these studies will elucidate how T cells and miR-25 may be linked to CRPS pathophysiology and how bone marrow may be affected, addressing a growing body of literature that supports a role for T cells in CRPS manifestation.

30. Validating Inflammation-related Genes as Potential Biomarkers for Personalized Treatment in Diabetic Foot Ulcers

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Inflammation and wound healing are complex, linked processes that are altered in nonhealing diabetic foot ulcers (DFU). Treatment of DFUs is challenging; wound assessments are subjective, and clinicians do not know whether the therapies they are recommending are promoting healing until months after initiation. New potential biomarkers enable more effective early diagnosis and support the advancement

of personalized medicine. Chronic inflammation is strongly associated with impaired wound healing in DFUs. Inflammation can be used as biomarkers for prediction and monitoring of healing status and holds potential to provide actionable information to guide treatment decisions. Our previous results have demonstrated that the Inflammation Index, a measurement of gene expression from 4 early-stage pro-inflammatory gene markers (IL1b, CD80, CCR7, VEGFA) to 3 late-stage inflammation-resolution gene markers (MRC1, TIMP3, PDGFB) over time, is highly predictive of healing trajectory. The Inflammation Index utilizes qRT-PCR to measure gene expression of the biomarkers in debrided wound tissue and is an indirect measurement of the wound's healing stage. Because the measurement technique uses debrided wound tissue, which can be highly degraded, we first investigated reliability and reproducibility of gene expression measurement as a function of sample degradation and purity using lab-prepared samples of primary human macrophages cultured on collagen scaffolds. We found that by processing the gene expression values as ratios to either a composite housekeeping gene metric or as defined in the novel Inflammation Index, reliable and reproducible measurements could be obtained from samples with high levels of degradation (RIN 1.9) and contamination (260/280nm ratio 1.4). Next, we measured the Inflammation Index in DFU tissues collected from 44 subjects collected across 5 independent studies, including 14 subjects enrolled in the recently established national Diabetic Foot Consortium (DFC). A logistic regression model showed that the ratio of the Inflammation Index from samples taken at week 0 and week 4 predicts healing outcomes after 12 weeks. The model achieved a cross-validation accuracy of 89%, with an area under the ROC curve (AUC) of 0.87 (95% CI: 0.7552 - 0.9926, p < 0.0001). These findings suggest that with further validation in a larger cohort, the Inflammation Index could identify non-healing subjects earlier so that they could be treated with more advanced treatments.

31. Design optimization of STING-mimicking peptide-polyanion conjugates for ovarian cancer immunotherapy

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Targeting the stimulator of interferon genes (STING) pathway to activate the innate immune system has emerged as a promising approach in cancer therapy. However, traditional STING agonists often fail to produce a robust immune response in the clinic. There is evidence that STING activation in cancer cells in addition to immune cells can improve the immune response, but STING protein expression is lost in many human cancers, including ovarian cancer.

To overcome this challenge, we have developed a multivalent STING peptide-polyanion conjugate that mimics the multimeric active state of the STING protein, enabling direct activation of signaling proteins TBK1 and IRF3 when delivered to the cytosol. The conjugate structure consists of a polyanion backbone with multiple copies of peptide covalently bonded to the side chain, allowing for tunability of peptide display. The peptide sequence is a 20 amino acid fragment of the STING protein's C-terminal tail containing the interaction motifs for TBK1 and IRF3, which we have shown can activate the same downstream signaling as the entire STING protein.

In this work, we demonstrate the development of design principles for this conjugate platform to determine how conjugate structure impacts efficacy and delivery via a lipid nanoparticle (LNP) carrier. We synthesized alkyne-functionalized carboxylate polymers using EDC carbodiimide cross-linker chemistry and conjugated the peptide using copper-catalyzed azide-alkyne cycloaddition to generate a library of conjugates with varying polymer molecular weight, peptide display valency, and backbone. The efficacy of each construct was evaluated using an in vitro IRF3 luciferase reporter assay to measure functional STING activation. Leveraging the negative charge of the conjugate material, each construct was formulated into LNPs and characterized based on encapsulation, transfection, and particle stability. Overall, we were able to optimize peptide-polyanion conjugate design and develop an LNP formulation with improved encapsulation, stability, and STING activation potency in vitro.

32. Integration of tissue-resident and circulating immune cells within a modular microphysiologic model of the cervix

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Microphysiologic systems (MPS) have been well established in engineering laboratories, but a important component of physiology that is often not included in these models is the immune response. The immune system plays a vital role in homeostasis and pathogenesis, and mechanistic studies should incorporate immune cells into MPS devices. However, the increased complexity that MPS provide requires an increase in expertise, decreasing the useability of the systems by the non-engineering community. Therefore, our goal was to create an accessible MPS model of the human cervix with functional tissue-resident and circulating immune cells. We first modified our easy-to-use standard insert-cassette MPS model to house a hydrated stromal layer able to incorporate resident immune cells. We differentiated THP-1 monocytes to macrophages and embedded them in a collagen-I hydrogel within the MPS. We completed the model by seeding epithelial and endothelial cells on either side of the stromal collagen-I hydrogel. To validate the function of the tissue resident cells, we stimulated the model with LPS and measured cytokine (IL-6, IL-1β, IL-8) response using ELISA with or without macrophages. An increase in cytokine expression was observed, validating functional response from embedded stromal immune cells. To validate proper circulating immune cell recruitment in response to a stimulus, we perfused human polymorphonuclear neutrophils (PMN) in the vascular channel and colonized the epithelium with Neisseria gonorrhoeae (Ng). We found that in response to epithelial infection, PMN were able to be recruited from the circulating vascular channel, migrate through the stromal hydrogel, and interact with the Ng at the apical surface of the epithelial cells. This user-friendly MPS enables study of resident and circulating immune cells in a cervix-relevant context, supporting mechanistic interrogation of infection, as well as screening of therapeutics. The modular design lowers the barrier for non-engineering labs and is readily adaptable to other mucosal tissues.

33. PD-L1 ligation on NK cells induces a metabolic shift from glycolysis to fatty acid oxidation, enhancing tumor infiltration and control

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PD-L1 blockade benefits even PD-L1-negative tumors, suggesting that non-tumor cells contribute to PD-L1 expression. Natural killer (NK) cells, vital mediators of innate immunity, vigorously express PD-L1 upon activation. We demonstrate that the ligation of PD-L1 on circulating and tumor-infiltrating NK cells with the therapeutic anti-PD-L1 antibody atezolizumab, soluble PD-1, or PD-1+ cells enhances NK cell-mediated tumor clearance via changes in metabolism, adhesion, and migration. PD-L1 engagement increases NK cell tumor infiltration via the CXCR3 pathway and cytoskeletal remodeling, supported by a metabolic shift from glycolysis to fatty acid oxidation (FAO). Loss of a key FAO enzyme, CPT1A, in NK cells abrogates the PD-L1-mediated anti-tumor effect, supporting a critical role for FAO in enhanced NK cell killing. The PD-L1-triggered shift away from glycolysis permits NK cells to remain highly effective at tumor killing in glucose-restricted TME. Taken together, PD-L1 ligation enhances NK cell cytotoxicity and tumor infiltration and contributes to NK resilience in challenging TME conditions, resulting in a more effective anti-tumor immunity.

34. Immune Checkpoint-Conjugated Hydrogels To Treat Psoriasis

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Autoimmune diseases, like psoriasis, are hallmarked by regulatory T cell (Treg) deficiency and increased autoreactive/pro-inflammatory T cell phenotypes. While conventional treatments rely on systemic immunosuppression, this precipitates infection and cancer risks. Treg cell therapies (adoptive cell transfer or in vivo induction) have been developed, but have issues with cell purity, Treg stability, precise delivery, and off-target effects. To address these limitations, we developed injectable hydrogels for local presentation of B7x (immune checkpoint) to enhance Treg abundance. Hyaluronic acid

hydrogels were functionalized with DBCO for anchoring azide-terminated B7x. Hydrogels underwent extrusion fragmentation to form microgels (diameter=155±53μm) and were centrifuged to form injectable granular hydrogels (shear-thinning assessed by rheology). Jurkat cells migrated into CCL21-loaded hydrogels in a dose-dependent manner, ensuring cell infiltration and survival. In imiquimod-induced psoriasis mice, mice received subq injection of hydrogel, gel+soluble B7x, or B7x-conjugated gel. Imiquimod was applied, and Psoriasis Area Severity Index (PASI) scores were assessed daily. T cell populations (Th17, γδT17, Treg) in the skin and hydrogels were examined (flow cytometry) on day 6. B7x or B7x-conjugated hydrogels reduced PASI scores, relative to imiquimod-treated mice; relative weights were recovered by sB7x. B7x-conjugated hydrogels increased Treg abundance and reduced pro-inflammatory T cell subsets (Th17/γδT17) locally in the skin, with no significant changes in the hydrogel. The local ligand conjugation system is promising for overcoming limitations of soluble factors in Treg induction, with potential applications in treating autoimmune dysfunction, organ rejection, and tissue repair.

35. Lymphatic endothelial cell-targeting nanocarriers for modulating vaccine-induced immunity
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Vaccine-induced immunity is limited by the decline of memory cell numbers over time. Vaccines targeting lymphatic endothelial cells (LECs) may help address this limitation. The lymphatic vascular system is critical for coordinating immune responses to diseases. LECs line the lymphatic vessels and lymph nodes, and are uniquely positioned to interact with antigens and immune cells to modulate the immune response. LECs are known to capture, store, and present antigen to T cells, which preferentially generate a memory-like T cell phenotype that rapidly proliferates upon antigenic rechallenge. These processes may help improve vaccine-induced immunity by biasing towards memory T cell generation. However, no vaccine platforms have been developed to specifically target LECs, limiting understanding of their immunomodulatory properties.

To address this, we developed LEC-targeted lipid nanoparticle (LNP) vaccines by screening several peptide ligands targeting surface receptors selectively expressed by LECs. Peptides were synthesized by Fmoc solid-phase peptide synthesis and purified by HPLC. Using thiol-maleimide chemistry, peptides were conjugated to LNPs containing EGFP mRNA (EGFP LNPs). To evaluate LEC targeting in vitro, LECs were treated with unconjugated or peptide-conjugated EGFP-LNPs. EGFP transfection was measured by live-cell microscopy and flow cytometry. In vivo, EGFP-LNPs were injected intradermally in mice, and skin-draining lymph nodes were analyzed by flow cytometry and immunofluorescent imaging. We identified several LEC-targeting peptide-conjugated LNPs that increased eGFP transfection of LECs over unmodified LNPs in vitro and in vivo. Unmodified or LEC-targeted LNPs were next loaded with OVA mRNA (OVA LNPs). In mice vaccinated with LEC-targeted OVA LNPs, OVA-specific T cells upregulated markers associated with a memory T cell response compared to unmodified OVA LNPs. Here, we show the development of LEC-targeted LNPs, and the potential to modulate the vaccine response towards a long-lived memory T cell response. Future studies will assess the functional response and efficacy of LEC-targeted LNP vaccines.

36. Investigating the impact of donor variability in fibrosis targeting cell therapy
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Pulmonary fibrosis is a progressive and fatal disease. Currently, there are no treatments that can reverse fibrosis. Macrophages are innate immune cells capable of degrading the collagen that constitutes fibrotic tissue, making them a good candidate for cell therapy. However, macrophages are plastic and become profibrotic in response to the fibrotic microenvironment found in pulmonary fibrosis. Furthermore, variability in donor biology due to natural variation or other factors, like age, complicate the consistency and efficacy of cell therapy. In this study we generated a cell therapy that locks macrophages into a fibrosis resolving phenotype by loading the cells with poly lactic-co-glycolic acid (PLGA) microparticles containing the anti-inflammatory corticosteroid dexamethasone (DexMP-macs).

Macrophages engulf the microparticles by phagocytosis and the dexamethasone is released over time, resulting in a phenotype that displays fibrosis resolving characteristics, even in the presence of the proinflammatory, lipopolysaccharide (LPS) and interferon γ (IFNγ), and profibrotic stimuli, interleukins 4 and 13.

Messenger RNA sequencing was performed on DexMP-macs generated from 20 healthy human donors, 9 under age 47 and 11 over age 65. Macrophages were cultured with DexMP for 4 hours at a dose of 100ug per million macrophages. Then DexMP-macs were stimulated with LPS (100ng/mL), IFNγ (100ng/mL), IL4 (40ng/mL), and IL13 (20ng/mL) for 48 hours. While we didn't find significant differences in gene expression due to age, we did observe a high degree of donor-to-donor variation, highlighting the importance of characterizing donor variability when developing cell therapies. Despite high variation, DexMP-macs upregulated the expression of several cathepsins, which are lysosomal proteases that degrade internalized collagen, along with other key genes associated with a fibrosis resolving phenotype, including MFGE8 and APOE, when compared to unmodified macrophages and macrophages loaded with microparticles lacking dexamethasone. In conclusion, this study demonstrates that DexMP-macs take on a phenotype that displays fibrosis resolving characteristics.

37. The Immune Landscape of Melanoma in People Living with HIV
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People living with HIV (PLWH) diagnosed with melanoma have consistently worse clinical outcomes than HIV-negative individuals (PLw/oH), even in the era of antiretroviral therapy (ART). To investigate contributing factors, we analyzed electronic health records from 922 PLWH and 334,972 PLw/oH with melanoma. Our findings confirm prior smaller-scale studies to show that PLWH have nearly a fourfold increase in mortality. We also found that PLWH are more likely to be diagnosed at younger ages, experience treatment delays, and have a higher incidence of brain metastases.

To identify potential biological drivers of these disparities, we performed spatial immune transcriptomics on melanoma tumors (n=11). This revealed a more immunosuppressive tumor microenvironment in PLWH, marked by elevated expression of immune checkpoints (e.g., PD1, LAG3, CTLA4) and reduced antigen presentation (e.g., HLA-DRB, B2M), with distinct spatial differences between tumors versus the tumor microenvironments. We validated these findings using multiplex immunofluorescence (n=15 PLWH, n=14 PLw/oH), which showed an exhausted CD8+ T cell phenotype, particularly PD1intLAG3- and PD1intLAG3+ subpopulations, and a significant accumulation of myeloid-derived suppressor cells (MDSCs; CD11b+ HLA-DR- CD33+).

To further investigate how CD8+ T cells and MDSCs interact in limiting melanoma cell killing, we developed a novel in vitro triple-culture system. Using this platform, we are studying MDSC-mediated immunosuppression and comparing native T cell responses from PLWH and PLw/oH to melanoma. We have successfully isolated the rare MDSC population (<1% of PBMCs), optimized the culture system, and initiated functional studies in PLWH, with results forthcoming. Together, these findings highlight that both biological and clinical factors contribute to the disproportionately poor outcomes for PLWH with melanoma. Our work uncovering the unique immunological landscape of melanoma tumors in PLWH, combined with our novel functional co-culture, lays the groundwork for targeted therapeutic strategies to enhance immune responses and improve survival outcomes for this high-risk population."

38. Harnessing Heligmosomoides polygyrus for immune modulation and regenerative therapies
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There are profound deficits in how humans, particularly adults, recover from tissue damage. The alternative to productive healing is dysfunctional scarring, which leads to loss of function, fibrosis, and chronic inflammation that accelerates with aging. The immune system is the first responder to trauma,

yet this response and its capacity to orchestrate healing have been largely ignored. Therefore, we aim to leverage potent helminthic stimulation of Type 2 immunity to develop new regenerative immunotherapies. Parasitic helminths have coevolved with humans over millennia, intricately refining and developing an array of mechanisms to suppress or skew the host's immune system, thereby promoting their long-term survival. In this present work, we advance the concept of helminth-derived regenerative immunotherapies by exploring Heligmosomoides polygyrus (H. polygyrus, Hp), one of the most potent Type 2 immune-stimulating helminths. We hypothesize that H. polygyrus regenerative formulations will serve as potent Type 2 stimulators that can directly form lipid nanoparticles (LNPs) to improve tissue repair. H. polygyrus has a unique life cycle and only infects mice; it causes limited morbidity, enabling safe cultivation and potentially large-scale production. To generate Hp-derived regenerative immunotherapies, we propose to 1.) evaluate the impact of H. polygyrus infection on distal muscle tissue after injury, 2.) develop a Type 2 regenerative immunotherapy formulation from Hp extracts, 3.) formulate Hp extracts into LNPs, and 4.) evaluate the regenerative capacity of Hp-derived LNPs. Preliminary studies show that H. polygyrus induces persistent immunological changes distal to infection sites even after clearance. We also observed that Hp egg extracts induce a strong Type 2 response both in vitro and in a volumetric muscle loss injury model while reducing inflammation and fibrosis. Finally, we show that Hp egg extracts can be formulated into LNPs without any additional components and demonstrate their ability to stimulate a Type 2 response in vitro.

39. Unraveling the Mechanobiology of Inflammation in Periodontal Health and Disease

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Objective- Periodontal disease is linked to ECM disruption by host and microbial proteases, but the bio-mechanical changes in tissue resulting from this disruption have not been investigated. This work characterizes stiffness- responsive behavior of gingival fibroblasts (GFs) and its interaction with monocytes by using a three dimensional (3D), mechanically tunable artificial gingival ECM.

Methods- A gingival ECM mimicking hydrogel model is developed with tunable stiffness, replicating both healthy and diseased gingival ECM. Stiffness based responses of GF and its cross talk with monocytes were studied constitutively and after TLR2 activation.

Results- Second harmonic generation (SHG) imaging on human gingival tissues showed extensive collagen degradation in areas with high inflammatory cell infiltrates. Gingival hydrogel model showed physiological relevance with native tissue with similar range of G' and tan(delta). GFs in softer matrix showed fibroblast migration with uniaxial protrusions tipped with small lamellipodial blebs, which were inhibited with increasing matrix stiffness and inhibitors of non-muscle myosin II and Rho-ROCK pathway. Stiff ECM upregulates collagen matrisome related pathways, TGF-beta signaling and TIMP3 expression, downregulates MMPs and noncanonical NF-κB signaling in GFs. Stiffer Matrix attenuates TLR2 mediated secretion of IL-6, IL-8 and CCL-2 and shows stiffness-dependent reduction in total nuclear volume of GFs with transcriptional upregulation of pathways associated with ECM-cytoskeletal-nuclear axis. DNA methyltransferase inhibition abolished the impact of matrix stiffness on gingival fibroblasts spreading and IL-6 secretion. Co-culture studies showes GFs in stiff matrix promote dendritic cell differentiation of myeloid precursors and increased expression of co-inhibitory PD-L1. Appropriate statistical tests were performed to assess significant differences between groups.

Conclusion- ECM stiffness differentially regulates gingival fibroblast-monocyte crosstalk and immune responses where stiffer matrix are critical in maintaining tissue homeostasis.

Significance - This work provides key insights into the mechanobiological mechanisms underlying periodontal disease pathogenesis which will inform identification of mechano-immune targets and development of novel therapeutics.

40. 3D Anatomical Reconstruction of the Lymph Node for Spatial Pharmacokinetic Modeling

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Lymph nodes (LNs) play a critical role in adaptive immunity and systemic homeostasis. However, LNs can also play a central role in disease persistence. Therefore, targeted drug delivery to the LN is a growing area of interest, although the tightly regulated microenvironment and compartmentalization of the LN hinder effective drug penetration. Pharmacokinetic (PK) models are often used to test and define the LN-penetrating properties of different drugs; however, current PK models lack the resolution needed to capture spatial drug heterogeneity and its impact on disease persistence. To overcome this, high-fidelity 3D spatial information is needed to quantify and computationally predict cellular organization and drug distribution patterning. Our objective was to develop a pipeline for processing, imaging, segmenting, and 3D anatomical reconstruction of LNs. Murine LNs were harvested, sectioned via vibratome at a thickness of 100 μm, and stained for the lymphatic sinuses (LYVE-1), vasculature (CD31), and high endothelial venules (HEVs) (MECA-79) for fluorescence confocal microscopy. A random sample of images was manually segmented to serve as ground truth. Next, we utilized this dataset to train a U-Net convolutional neural network for segmenting the lobule and HEVs, achieving an overall accuracy, intersection-over-union, and F1-score of 0.9916, 0.9838, and 0.9914, respectively, on the testing set. The trained model was used to segment the remaining image slices. Adjacent images were aligned using Euclidean transformations, spline-interpolated to increase resolution in the z-plane, and converted into a point cloud before meshing. This resulted in a computational mesh of the lobule and HEVs to be used for modeling fluid and cell dynamics and spatial drug distribution patterning in the LN. In the future, our goal is to use this pipeline to reconstruct multiple LNs to investigate the effect of architecture variability on spatial drug transport and disease progression.

41. Role of Dimensionality and ECM Composition in Macrophage Polarization
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Macrophage phenotype and function are tightly regulated by cues from their surrounding extracellular matrix (ECM), including mechanical properties, biochemical composition, and spatial architecture. In this study, we explore how dimensionality and matrix composition influence the polarization and metabolic programming of RAW 264.7 macrophages. Cells were first treated under three priming conditions: unconditioned (UC), M1-polarized (LPS + IFN-γ), and M2-polarized (IL-4 + IL-13) and subsequently cultured in 2D tissue culture plastic, or in 3D hydrogels composed of either type I collagen or lung derived decellularized ECM (dECM). Gene expression analysis via qPCR revealed that 3D environments broadly upregulated both M1 and M2 markers in unconditioned and M1-primed macrophages, suggesting an amplification of transcriptional activity independent of priming. Specifically, UC macrophages in 3D collagen showed increased expression of anti-inflammatory genes (e.g., VEGF, CCL-22, IL-10), indicating a possible shift toward an M2-like phenotype. M2-primed cells, however, exhibited a general downregulation of polarization markers in 3D, except for paradoxical elevation of TNF-α. These results highlight the context dependent plasticity of macrophages in response to 3D matrix cues. Ongoing work incorporates Seahorse extracellular flux analysis to correlate transcriptional findings with metabolic shifts in glycolysis and oxidative phosphorylation. Additionally, dECM hydrogels are being investigated as a more physiologically relevant scaffold to evaluate how native lung ECM composition further modulates immune and metabolic responses. Together, these findings demonstrate that both dimensionality and ECM composition significantly influence macrophage behavior and underscore the importance of 3D systems for accurately modeling immune function in vitro.

42. Hyperactive Rac enhances macrophage phagocytosis in immunodeficiency and cancer
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Rac2, a hematopoietic-specific GTPase, plays a critical role in phagocytic functions, including fluid uptake, apoptotic cell clearance, and pathogen elimination. Loss-of-function mutations in Rac2 impair neutrophil, macrophage, and lymphocyte functions, leading to immunodeficiency. Patients with germline gain-of-function mutations, particularly the Rac2E62K variant, present with recurrent respiratory infections and T cell lymphopenia. Interestingly there are no defects in T cell development or maturation, leaving the cause of T cell lymphopenia a mystery. Recent work in our lab reveals that hyperactive Rac is sufficient to drive live cell engulfment, a phenomenon we first described in Drosophila. We hypothesized that the Rac2+/E62K mutation might similarly promote aberrant phagocytic behavior in the patients, leading to immune cell loss. Using a Rac2+/E62K mouse model that phenocopies the human condition, we discovered that Rac2+/E62K macrophages exhibit hyperphagocytic activity, engulfing significantly more T cells than wildtype macrophages. Our findings indicate that hyperactive Rac2 acts in a cell-autonomous manner to promote phagocytosis and T cell activation, which may explain the lymphopenia and immune dysregulation observed in Rac2+/E62K patients. Additionally, we found that Rac2+/E62K macrophages also have a non-autonomous effect and can stimulate wildtype macrophages to eat more cellular targets. Importantly, this work translates a phenomenon discovered in the Drosophila into a mammalian context, providing a novel cellular and molecular explanation for an otherwise mysterious rare immunodeficiency in patients. Finally, we propose that though harmful as a germline mutation, we can harness this Rac-mediated behavior in macrophages to enhance cancer cell killing in CAR macrophage (CAR-M) and antibody-based immunotherapies.

43. CAR-TIME-induced antigen loss

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FDA-approved chimeric antigen receptor T cell therapies (CARTs) have revolutionized the treatment of blood cancers. Yet none have been successful in clinical trials for “solid” tumors, such as colorectal (CRC), gastric (GC), and esophageal (EC) cancers, which are leading causes of cancer deaths. In that context, guanylate cyclase C (GCC) is a membrane receptor expressed by nearly all CRCs, as well as many GCs and ECs, and is a leading immunotherapy target. While GCC-directed CART (GCC-CART) can effectively control GCChigh CRCs in preclinical studies, further investigation revealed poor efficacy in GCClow CRC, GC, and EC models, as well as reduced GCC levels in cancer cells following GCC-CART. Antigen escape is a known contributor to CART failure due to the selection of CART-resistant cancer cells with low antigen levels. Current understanding of antigen escape mechanisms includes pre-existing antigen-negative tumor clones, mutations or alternative splicing of antigen genes, subcellular antigen redistribution, lineage switching, epitope masking, and trogocytosis-mediated antigen loss. However, bystander effects on antigen escape by the CAR-T immune microenvironment (CAR-TIME) have not been reported. Our preliminary data reveals CAR-TIME-induced GCC reduction independent of direct CART contact with CRC, GC, and EC cells. We found reduced GCC mRNA and protein, decreased GCC protein synthesis, and accelerated GCC degradation in cancer cells exposed to active CAR-TIME. Transcriptomic data suggests unfolded protein response and endolysosomal degradation as mechanisms for reduced GCC levels within activated CAR-TIME. Moreover, reduced GCC levels in bystander cells result in escape and GCC-CART failure. Our study reveals a novel CAR-TIME-induced antigen escape mechanism independent of direct cell contact between CART cells and cancer cells. This finding might be generalizable to other immunotherapeutic targets and other modalities of immunotherapies for CRCs. Moreover, the insights from understanding this escape mechanism might be targeted to improve the efficacy of various CART therapies.

44. Cryoshocked T Lymphocytes (CSTLs) for Targeted Immunomodulation of the Lymph Node

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Failure of cancer therapies is driven by a myriad of factors; one of which is the major role immune tolerance plays within the tumor microenvironment and associated lymphatic tissues. The cyclic GMP–

AMP synthase–stimulator of interferon genes (cGAS–STING) pathway is a promising target for reversing this immunosuppression by activating dendritic cells, enhancing antigen presentation, and promoting robust anti-tumor T cell responses. However, clinical translation of STING agonists (STINGa) has been hindered by rapid systemic clearance, poor penetration into lymph nodes (LNs), and dose-limiting toxicities. To address these barriers, we have developed Cryoshocked T Lymphocytes (CSTLs), a T cell–mimetic drug delivery platform engineered to exploit endogenous lymphocyte trafficking for targeted delivery of STINGa to tumor-draining lymph nodes (TDLNs). CSTLs are derived from primary T cells and modified to enable encapsulation and release a range of payloads including c-di-GMP, a STING agonist. CSTLs demonstrated efficient drug loading and sustained release, achieving ~75% release over a 12 hour period in vitro. Following in vivo administration and compared to a free drug control, drug-laden CSTLs achieved a 700% increase to LN specific delivery by 8 hours. Finally, delivery of c-di-GMP in CSTLs to LNs resulted in an increased innate response and heightening of STING agonism in LN resident antigen-presenting cell pools. These results indicate that CSTLs can overcome key translational barriers for STINGa therapy by improving LN penetration, modifying the immune phenotype within TDLNs, and broadening the therapeutic window. This biomimetic platform holds promise for enhancing the efficacy and safety of immunomodulatory drugs across multiple cancer types and provides a strong foundation for future translational and clinical investigations.

45. Functional Comparison of Tolerogenic Dendritic Cells Induced by All-Trans Retinoic Acid and Dexamethasone

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Tolerogenic dendritic cells (tolDCs) regulate autoimmunity and are critical for immune homeostasis. Treatment-induced tolDCs can selectively inhibit pathogenic T cells without compromising protective immunity, making them a promising therapeutic avenue for autoimmune disorders. Both dexamethasone (Dexa) and all-trans retinoic acid (RA) have been widely used to induce the differentiation of tolDCs from bone marrow stem cells. However, the functional differences between RA-tolDCs and Dexa-tolDCs remain to be determined. Additionally, there are few studies that systemically evaluate the impact of growth factors and adjuvants on tolDCs phenotype and function. In present study, we attempt to determine the surface protein expression and function differences between RA-tolDC and Dexa-tolDCs through flow cytometry and T-cell coculture assays. Additionally, we systemically examine the impact of different growth factors and stimulating adjuvants on the induction of bone marrow-derived tolDCs. We show that FLT3L (Fms-like tyrosine kinase 3 ligand) and LPS (lipopolysaccharide) are the most effective combination for the induction of tolDCs according to the expression level of key surface proteins, including MHCII (major histocompatibility complex class II), PD-L1/2 (program death ligand 1/2). Moreover, RA show superior ability to induce the expression of MHCII and PD-L1/2 relative to Dexa, suggesting that RA-tolDCs are more effective in promoting antigen-specific immune suppression than Dexa-tolDCs. In a DC/T-cell coculture assays, we show both RA-tolDCs and Dexa-tolDCs suppress T-cell activation. Blocking PD-1 on T cells via antibodies partially ameliorates T-cell inhibition during coculture with both RA-tolDCs and Dexa-tolDCs, indicating that PD-L1/2 is involved in the T-cell regulation. Collectively, we have established an effective condition for tolDC induction and demonstrated the functional differences between RA-tolDCs and Dexa-tolDCs. This study supports the future development of tolDC-based antigen-specific immunotherapy.

46. A local anti-inflammatory niche for the treatment of chronic tissue healing via recruitment of anti-cytokine B cells

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Duke University

Chronic inflammation, driven by sustained myeloid cell activity and persistent IL-1β signaling, impairs the resolution phase of tissue repair by perpetuating dysregulated inflammation.1–3 As a central

upstream mediator, IL-1β establishes a self-reinforcing pro-inflammatory loop, maintaining macrophage activation and creating an environment that hinders effective tissue regeneration4–7. Although biologics have been approved to block IL-1β signaling, their clinical use is limited by short half-lives, the need for repeated administration, and the risk of anti-drug responses.4,7 Moreover, none are approved for use in wound healing, where existing treatments rely on debridement and passive dressings that fail to modulate underlying immune dysregulation.3,8 To address this issue, we developed the novel immunotherapy iMAP designed to elicit robust, autologous, and specific anti-IL-1β immune responses capable of mitigating excessive IL-1β signaling at target sites. The approach enables the recruitment of anti-IL-1β-specific antibodies and lymphocytes to target tissues via antigenic recall, promoting the generation of a localized anti-inflammatory niche. iMAP promotes a tolerogenic T cell phenotype, by shifting populations from pro-inflammatory (Th1/Th17) to anti-inflammatory (Th2/Treg), enriching pro-healing cytokines such as IL-10, IL-4, and TGF-β. iMAP treatment significantly reduced the recruitment of inflammatory myeloid cells, neutrophils, and macrophages. Moreover, iMAP treatment increased the number of CD206+Arg1+ macrophages and proliferative Arg1+ macrophages, both of which are known M2-like macrophages. Notably, iMAP treatment also reduced non-polarized macrophages and the expression of senescence markers like PD-1 and LAG-3, indicating that a local anti-inflammatory niche can restore the ability of these cells to perform their basic functions, which are known to be impaired under chronic inflammation. In addition, iMAP treatment significantly reduced IL-1a concentration at the injury site, while also reducing IL-1β, IFN-γ, and other pro-inflammatory cytokines, and significantly increasing IL-4 and VEFG. Finally, we confirmed that iMAP treatment significantly improved tissue regeneration, as measured by a standardized histological assessment.

47. Tailoring Polymeric Nanoparticles Properties for Enhanced Targeted Delivery to Macrophage Subpopulation

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Macrophages are central mediators of the innate immune response, distinguished by their phagocytic capacity. Depending on microenvironmental cues, they polarize into two phenotypic extremes: classically activated M1 or alternatively activated M2. This plasticity has profound implications for tissue homeostasis and disease pathogenesis. M1 macrophages drive inflammation and, when dysregulated, contribute to tissue damage and chronic inflammatory diseases. Conversely, M2 macrophages exhibit anti-inflammatory and reparative functions but can also promote tumor/cancer progression. Since these phenotypes often coexist in tissues, their populational and functional imbalance contributes to specific disease, highlighting the need for drug delivery systems that selectively target one phenotype to achieve therapeutic precision.

Nanoparticles (NPs) are promising candidates for such targeted strategies due to their tunable physicochemical properties. Inspired by studies highlighting the role of particles' physicochemical properties as intrinsic determinants of their cellular and biological interactions, we synthesized polymeric NPs of tunable sizes and charges via nanoprecipitation, systematically varying polymer (PEG-PLGA) and surfactant concentrations (PVA, CTAB, SDS). Characterization by TEM and DLS confirmed successful fabrication of NPs ranging from ~55 to ~900 nm in size and with surface charges spanning –42.13 to +25.67 mV. These formulations provided a platform to investigate preferential uptake by macrophage subsets.

In RAW 264.7 cells polarized to M1 (LPS + IFN-γ) or M2 (IL-4 + IL-13) states, PVA-stabilized NPs (~100 nm, –3 mV, neutral) showed preferential uptake by M1 macrophages. In contrast, SDS-stabilized NPs (~100 nm, –28 mV, negatively charged) were more efficiently internalized by M2 macrophages, as confirmed by flow cytometry and fluorescence microscopy. Having mitigated cytotoxicity associated with highly positive CTAB-stabilized NPs, our optimized formulation yielded a modest positive charge (~100 nm, +3 mV) with improved biocompatibility, enabling ongoing studies that systematically assess how NPs of similar sizes but different charges as well as NPs of different sizes, but similar charges influence macrophage-specific uptake.

Together, these findings highlight the potential of rationally designed NPs for selective macrophage targeting and underscore the need for validation in physiologically relevant models such as PBMC-derived macrophages."

48. Novel approaches for the treatment of melanoma in PLWH

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People living with HIV (PLWH) have experienced a significant decline in AIDS-defining cancers due to widespread antiretroviral therapy (ART). However, the incidence and mortality of non-AIDS-defining cancers, particularly cutaneous melanoma, continue to rise. PLWH diagnosed with melanoma have a higher melanoma-specific mortality compared to uninfected individuals, even when adjusted for stage and treatment, suggesting underlying biological interactions between HIV infection and melanoma progression. Using spatial transcriptomics on melanoma samples from PLWH, we identified a profoundly immune-suppressed, “cold” tumor microenvironment (TME) marked by myeloid-derived suppressor cell (MDSC) infiltration, T-cell exhaustion, fibrosis, and increased expression of immune checkpoints including PD-1 and LAG-3. Notably, ART does not normalize the abundance or function of MDSCs. To model these interactions, we developed the first immunocompetent “all-mouse” system of HIV and melanoma co-morbidity using the ecotropic-HIV (EcoHIV) virus. EcoHIV-infected mice display accelerated tumor progression, enhanced collagen deposition, and immunosuppressive TME features resembling those seen in PLWH. This model will overcome major limitations of existing preclinical systems by enabling the study of HIV and cancer interactions in fully immune-competent hosts. Using this model, we propose two therapeutic strategies: i) tailored immune checkpoint inhibitor (ICI) combinations informed by the distinct checkpoint expression landscape in PLWH, and ii) polarization of MDSCs toward a pro-inflammatory phenotype using a synthetic TLR2 agonist (CU-T12-9) delivered via a nanoparticle-hydrogel platform for sustained, localized release. Preliminary data support the efficacy of both approaches in reversing immune suppression and enhancing anti-tumor responses. This innovative mouse model offers a scalable and translationally relevant system to dissect HIV and melanoma comorbidity and evaluate rationally designed therapies. Our findings will provide a foundation for future clinical trials and contribute to a more inclusive and mechanistically informed approach to melanoma immunotherapy.

49. Modulating Macrophage Repair with Prostaglandin E2

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The role of Prostaglandin E2 (PGE2) in macrophage-mediated tissue repair is of growing interest, while building on its known pro-angiogenic functions. This study sought to comprehensively define how soluble PGE2 modulates macrophage phenotype across various simulated clinical scenarios in vitro. We investigated the effects of PGE2 when administered before, during, or after the polarization of mouse macrophages with either pro-inflammatory or pro-reparative stimuli to represent delivery in vivo. Across these conditions, preliminary results showed that PGE2 enhances a pro-regenerative phenotype. This was marked by upregulation of key markers like Arg1, CD163, and CD206. To contextualize these effects, we compared PGE2’s performance against Dexamethasone, a well-established drug used in our lab. The findings demonstrate that PGE2 is a driver of a pro-reparative macrophage phenotype, making it a promising drug for therapeutic strategies.

50. Predicting the long-term phenotype of human T cells from transcriptional networks during early signaling events

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CAR T cell therapy is revolutionizing the treatment of hematologic malignancies, enabling high remission rates in patients with otherwise refractory disease. Emerging evidence indicates that

transcriptional programs engaged during early CAR T cell signaling shape their proliferation rates, persistence, and resistance to exhaustion, which are key determinants of therapeutic success. Our experimental data show that stimulation using our biomaterial platform with DNA-scaffolded CAR-engaging particles (CAREp), which precisely control antigen and costimulatory signal presentation, drives greater CAR T cell expansion than conventional tumor cell (U87) stimulation. However, the transcriptional regulatory networks underlying these differential activation pathways remain unknown. Here, we built a pipeline that infers multi-layered gene regulatory networks (GRNs) to determine the transcriptional programs driving stronger CAREp proliferation and assess the impact of CD28 co-stimulation. We performed gene set enrichment analysis to identify condition-specific pathways and mapped transcription factors (TFs) linked to both upregulated genes and enriched pathways to define condition-specific master regulators. The regulatory TF-TF and TF-target gene networks with upstream signaling and epigenetic inputs were then inferred for these regulators. Using this approach, we found that CAREp stimulation activated MYC, a master regulator of proliferation, through core cell cycle TFs E2F1 and YY1. MYC activation promoted telomerase expression to drive cell cycle progression and telomere maintenance. However, tumor cell stimulation led to MYC repression by growth inhibitory TFs CEBPA and RFX1, with telomerase repressed by E2F1, suggesting regulatory wiring that likely limits long-term proliferation compared to CAREp. Our ongoing work includes examining TF activity levels and donor variability in the CAREp-specific regulatory programs. Our computational pipeline provides a framework to determine TF regulatory programs in T cells stimulated with distinct extracellular signals, enabling the identification of up- or downregulated TFs. Our research aims to enhance CAR T cell function and inform the design of next-generation vaccines and immunotherapies.

51. HoneyComb single-cell technology revealed transcriptional changes uniquely in monocytes from HAMP/TSP patients versus asymptomatic carriers of HTLV-1

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Infection with human T-cell lymphotropic virus type 1 (HTLV-1) causes HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a neuroinflammatory demyelinating condition of the spinal cord that is influenced by the peripheral inflammation. Identifying the elements that contribute to HAM/TSP development can offer useful insights into this disorder. Therefore, we performed single cell sequencing of total PBMCs from the asymptomatic carrier (AC) of virus and HAM/TSP patient by utilizing innovative HoneyComb technology to determine differential gene expression patterns between two groups. Upon extensive cellular and gene analysis, we observed significant differences between AC and HAM/TSP patient albit, only in monocytes, which have been the least explored cell type in HTLV-1 pathogenesis thus far. A total of 159 genes were identified to be differentially expressed by HAM/TSP monocytes of which 38 genes were upregulated, and 63 genes were downregulated. The Ingenuity pathway analysis (IPA) revealed that these genes belonged to various pathways such as macrophage classical activation signaling, IL-10 signaling, multiple sclerosis signaling, granulocyte adhesion & diapedesis signaling, and pathogen induced cytokine storm signaling pathways. Some of which are highly relevant to HAM/TSP pathogenesis (i.e. macrophage signaling and cytokine storm signaling pathway) while others (Multiple Sclerosis signaling, granulocyte adhesion and diapedesis signaling pathways) have never been studied in this context, which makes result of this analysis significant and innovative. Sequencing data has been validated into a large cohort of HTLV-1 infected patients from the endemic regions of Brazil utilizing samples from a longitudinal clinical trial with Dolutegravir, an antiretroviral drug. As expected, heightened expression of several inflammatory markers was suppressed upon the treatment in HAM/TSP patients confirming potential role of newly identified genes in the disease pathogenesis.

52. Development of TCR-T cell Therapeutics for Cancer Immunotherapy

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Adoptive cell transfer (ACT) therapy is a promising approach for cancer immunotherapy. However, it had limited success in solid tumors. Early attempts took advantage of the patient's tumor infiltrating lymphocytes (TILs) and provided mixed results, showing promise for the treatment of some cancers. However, this avenue relies on pre-existing tumor-specific T-cells of sufficient quality. To overcome this limitation, we redirect peripheral blood T-cells to target tumor cells by delivering genes encoding α and β TCR chains with the specificity of interest using a viral vector. However, this approach resulted in lower cytolytic activity compared to high-quality patient-derived CD8 clones. This loss of cytolytic efficiency can be attributed to semi-random insertion of the TCR genes into the genome and α/β chain mispairing with endogenous TCR, resulting in a heterogenous transgenic TCR-T cell population with varying levels of the TCR expression (D-endo-TCR) and reduced cancer-specific cytolytic activity. To address these problems, we used CRISPR-CAS9 editing to knock out endogenous α/β-TCR genes and virally transduced transgenic TCR-T cells (D-KO-TCR). This approach produced transgenic TCR T-cells specific for a cancer antigen of interest and outperformed regular virally transduced transgenic T-cells in both sensitivity and magnitude of response. To further improve the quality of TCR-T cells, we used CRISPR-CAS9 knock-in approach to insert transgenic TCR genes, specifically into endogenous TCR locus. This approach resulted in consistently high levels of TCR expression in T cells due to the natural maintenance of endogenous TCR locus in the open state that supports continuous transcription. A comprehensive comparison of these design strategies will provide crucial information for transgenic TCR-T cell-based therapy and new insight into TCR signaling.

53. Engineering Immunostimulatory Nanoparticles for Antiviral Defense against Influenza A Infection

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Influenza is a leading cause of mortality in the US and internationally; in non-pandemic years, influenza accounts for 3–5 million annual cases of hospitalization and 250,000–500,000 deaths globally while in pandemic years. Given human influenza A virus (IAV) can undergo genome reassortment with IAV from different species, new classes of broad acting prophylactic are needed. Poly(IC), a synthetic double stranded RNA, contains the same pathogen associated molecular patterns (PAMPS) as many viruses, allowing it to activate the same pathogen recognition receptors (PRR). This temporarily stimulates a heightened antiviral state through production of type 1 and 3 interferon (IFN). However, due to stability and toxicity concerns, poly(IC) has not been clinically implemented as a treatment to induce local immunity at vulnerable sites. To address its inherent limitations, we formulated polyplexes using polyethylene imine (PEI) and polyethylene imine-polyethylene glycol (PEI-PEG) copolymer to condense poly(IC) into nanoparticles to minimize dosages, bypass biological barriers and enhance bioavailability in target cells. Our particles, named Host Immunostimulatory Nanoparticles for Antiviral Responses (HINAR), are within the hydrodynamic size (178.1± 105.3 nm) and zeta potential (-16.1 ± 1.2 mV) ideal for intranasal delivery as a prophylactic treatment against respiratory infection. We found these nanoparticles are stable and can efficiently release poly(IC) based on heparin displacement assays. HINAR treatment can also produce detectable levels of type 1 and 3 IFN in vitro in A549 cells as well as in differentiated bronchial cells grown at an air liquid interface (ALI). We also tested HINAR pre-treatment in an influenza challenge model using ALI cultures and found reduced infection based on nucleoprotein staining. Future studies are planned to quantify effect on a co-culture of airway epithelial and immune cells. The proposed research will develop another tool to mitigate the IAV crisis and potentially other respiratory virus induced crises.

54. Extracellular Matrix Hydrogel Reduces Fibrosis In A Canine Model Of Volumetric Muscle Loss

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Volumetric muscle loss (VML) is a severe pathological condition involving the acute loss of substantial skeletal muscle mass, leading to chronically impaired function due to injury beyond the body's endogenous regenerative capacity. While present in both civilian and military populations, VML disproportionately affects military personnel. From 2001 to 2013, 77% of 14,500 evacuated military members sustained musculoskeletal injuries, and 65% of those discharged with severe orthopedic trauma experienced VML. Additionally, 8% of evacuees with multiple injuries reported VML-related disability. The lifetime cost per affected individual ranges from $340,000 to $440,000, excluding medical expenses and lost income. No standard surgical or regenerative therapy currently exists, partly due to insufficient understanding of VML's progression and the limitations of rodent models in mimicking human healing. This study explores the use of extracellular matrix hydrogel (ECM) as a therapy for VML using a canine model, which closely mimics human healing Researchers surgically induced a partial-thickness muscle defect (50–75% mass loss) in the biceps femoris using a standardized template. Biopsies at days 3, 7, 14, 21, and 42 post-injury evaluated healing through imaging, histology, and immunohistochemistry. Muscle progenitor cells, macrophages, and fibroblasts were identified using markers (Pax7, IBA-1, Mac387, CD163, α-SMA), with fibrosis assessed by Masson's trichrome staining. When applied thrice during healing, ECM treatments showed promising benefits: a 1.8-fold decrease in myofibroblasts at days 3 and 7, a 2.7-fold increase in satellite cells at day 14, and a 1.7-fold reduction in pro-inflammatory macrophages at day 7. By day 42, there was significantly reduced scar tissue depth. A pilot study showed that combining with ECM and the anti-fibrotic agent IDT-1 provided significantly enhanced muscle regeneration over fibrosis. While collagen maturity was unaffected, these findings underscore ECM's promise as a regenerative therapy and validate the canine model for translational VML research.

55. Autoantibody-based bispecific T-cell engagers as personalized cancer immunotherapy
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Despite the overexpression of tumor-associated antigens (TAAs), tumorigenesis is evolutionary and TAAs are not ubiquitously or uniformly expressed by tumor cells, resulting in high antigen repertoire heterogeneity within and between tumors. This variability makes it challenging to target tumors with a single biomarker approach, as subpopulations of non-expressing cells continue to proliferate. The humoral response raised in the tumor microenvironment (TME) results in the production of TAA-specific autoantibodies (AAbs) with high TME B-cell infiltration. However, AAbs alone do not control tumor growth but can be harnessed to localize tumor-cell killing agents to TAAs. We have discovered that tumors contain therapeutic quantities of bound, tumor-targeting autoantibodies, and we have developed a method to specifically extract functional tumor-bound AAbs from a resected tumor. We have validated our methods with multiple syngeneic Balb/c tumor models and 4 different types of human tumors. We have conjugated these tumor-binding AAbs to a cytotoxic CD3 recruiting domain to generate a T-cell redirecting AAb (TRAAb). We additionally present a formulation of TRAAbs that can be administered locally in the tumor resection cavity as a therapeutic agent. We believe that resected tumors have high potential to generate AAb-based therapies as a class of personalized immunotherapies.

56. Engineering Immune Tolerance through a Novel Strategy for Dendritic Cell Modulation
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Dendritic cells (DCs) are key regulators of immune homeostasis and central targets for antigen-specific tolerance induction in autoimmune disorders and transplant medicine. Our work integrates two complementary immunoengineering strategies—Push/Pull Immunomodulation (PPI) and lymph node-targeted liposomal delivery—to induce tolerogenic DCs (tolDCs) and promote regulatory T cell (Treg) expansion. Previously, we identified three optimized PPI combinations (PPI-1, 7, and 9) capable of inducing tolDCs from murine bone marrow-derived DCs (BMDCs) through large-scale high-throughput screening of combinatorial immunosuppressant and TLR agonist libraries, leading to robust antigen-specific Treg induction.

To enhance translational relevance, we evaluated these formulations in human monocyte-derived DCs (moDCs) differentiated from peripheral blood mononuclear cells (PBMCs). Compared to 6 clinically relevant tolerogenic agents, PPI-treated moDCs exhibited superior expression of PD-L1 and BTLA, enhanced longevity (>10 days), and sustained tolerogenic phenotypes. PPI-9–treated moDCs specifically generated CD25hi Tregs upon stimulation with antigenic peptide pools, but not with nonspecific CD3/CD28 stimulation, demonstrating antigen-specific immune modulation. Transcriptomic analysis further revealed distinct gene expression signatures in PPI-tolDCs, with upregulation of inhibitory cytokines and survival pathways compared to both natural and conventional induced tolDCs. We also developed liposomal PPI formulations for in vivo delivery. Together, these findings support a modular strategy for tolDC immunomodulation and lay the groundwork for the development of tolerogenic therapies in translational clinical studies.

57. INFLAMMATION-RESPONSIVE MICROGELS FOR DRUG DELIVERY IN CHRONIC INFLAMMATION MANAGEMENT

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Chronic inflammation is a prolonged, low-grade immune response that can lead to diseases with varying severity, such as rheumatoid arthritis and ulcerative colitis. For chronic-inflammation management, bolus dosage or sustained-release of anti-inflammatory drug like corticosteroids are clinically approved. However, these drug delivery methods could not correlate with inflammation severity, resulting in earlier depletion of drug reservoir rendering it insufficient for management of subsequent inflammation flares. Previously, our lab had developed a bulk hydrogel platform that could release drug in response to pro-inflammatory protease. Herein, we developed a drug-conjugated microgel system with high loading capacity and injectability, which can be locally administered and triggered by to release anti-inflammatory drug corresponding to different pro-inflammatory protease levels. During in vitro cyclic drug release test, which exposes our hydrogel periodically to alternating protease concentrations to mimic real-life chronic-inflammation disease flaring behavior, microgels exhibited more sensitive cyclic release behavior compared to bulk gel. Our in vitro results also demonstrated that releasate of microgel in response to MMP-9 digestion, retained its anti-inflammatory effects in inhibiting the secretion of tumor necrosis factor-α (TNF-α) from pro-inflammatory RAW 267.4 macrophages. Furthermore, subcutaneously injected microgels in immuno-competent SKH1-E mice suppressed TNF-α secretion induced by co-injected non-degradable polystyrene (PS), which stimulates localized immune activation in a model of subcutaneous inflammation. Together, our findings suggest that this drug-conjugated microgel is a promising platform with sensitive cyclic release kinetics and anti-inflammatory management efficacy.

58. Engineered HIV-Capturing Liposomes to Facilitate Immune Uptake

Ted Keunsil Kang, Peter Deak
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Despite over four decades of HIV research, a cure remains elusive due to the virus’s immune evasion strategies, including latent reservoir formation, high mutation rate, and extensive glycosylation. These features sustain infection, limit therapeutic efficacy, and hinder vaccine development.

We developed the Nanotrap Therapeutic Vaccine (NTV) to elicit HIV-specific adaptive immunity. NTV comprises (1) a bulky lipid forming the liposomal structure, (2) immune-stimulating adjuvants encapsulated in the core, and (3) a chemically synthesized lipid that binds gp120 for selective viral capture. The targeting component, CJF-III-288, an indoline-based HIV fusion inhibitor, was conjugated with various amino acids, PEG spacers, and lipid tails via solid-phase peptide synthesis to create three NTV-lipid candidates. Constructs were characterized by HPLC and MALDI, and gp120-binding was assessed by surface plasmon resonance (SPR). Liposome formation and adjuvant loading were analyzed by DLS and HPLC.

SPR confirmed that NTV-lipids retained gp120-binding activity, with the three-lysine conjugate showing affinity comparable to CJF-III-288. Flow cytometry and DLS demonstrated NTV binding to gp120-expressing cells and pseudotyped HIV. NTV did not block HIV infection in GHOST cells but did not interfere with ART efficacy. In vivo, NTV bound circulating HIV particles, was preferentially taken up by dendritic cells, and exhibited no major side effects.

NTV presents a novel liposomal platform for multivalent HIV capture and delivery to antigen-presenting cells, aiming to induce robust HIV-specific adaptive immunity. Current work focuses on evaluating in vitro and in vivo immunostimulatory activity and characterizing the quality and quantity of the elicited immune responses.

59. Loss of Lamin-A/C Enhances Anti-Tumor Macrophage Function
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Macrophage-based immunotherapies have been found to initiate acquired immunity against solid tumors, but face challenges due to poor infiltration and immunosuppressive microenvironment. Clinical data has sometimes linked clusters of tumor-associated macrophages (TAM) with improved patient survival, and chimeric antigen receptor macrophages (CAR-Ms) similarly form clusters in vivo. We find macrophage clusters form under maximal phagocytic conditions, where they cooperatively disrupt tumor cell adhesion, and we observe that M1 macrophages increase adhesion receptors, reduce actomyosin factors, and form clusters even without phagocytosis. Decreased cortical tension generally relates to downregulated SRF-target genes and low levels of nuclear lamin-A,C – which we hypothesized facilitates phagocytosis. Using conditionally immortalized macrophages (CIMs), we generated lamin-A,C knockouts that cluster more on low-adhesion substrates, express less vinculin and α-actinin, and phagocytose more cancer cells. Large deformations of the nucleus were also evident, consistent with a softened nucleus. Further combining lamin-A,C and SIRPα deletion in CAR-Ms can further enhance tumor clearance by lowering cortical tension for facile disruption of tumor cell adhesion without the CD47/SIRPα “Don’t Eat Me” signal. Upregulation of adhesion and M1 genes (ITGAL, ICAM1, MHC-II) provide a phenotype well-suited for antigen presentation and tumor immunity. Studies in 3D tumoroid models document infiltration and clustering, while constrained migration assays examine movement through restrictive environments. Targeting nuclear mechanics may thus be broadly leveraged to potentiate immune effector cells for treatment of solid tumors.

60. Comorbidity-driven immune dysregulation as a driver of Long COVID heterogeneity
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Long COVID (LC) or Post-Acute Sequelae of COVID-19 (PASC) affects ~6% of people infected and is defined as the chronic condition that is manifested by highly heterogenous symptoms and persists for at least three months after the SARS-CoV-2 infection. We hypothesize that the heterogeneity in LC symptomatology, severity and persistence is mediated by pre-existing immune alterations in the host environment i.e. comorbidity-associated cytokine and metabolite profiles.

We quantified 29 cytokines in plasma (1 month–3 years post–symptom onset) from participants varying in LC and comorbidity status, including lung disease (LD), and who segregated into five symptom clusters (neurological, musculoskeletal, systemic, gastrointestinal, and cardiopulmonary). Using ultrasensitive Meso Scale Discovery platform we found LD to be a greater driver of cytokine signatures than LC. Nine cytokines (M-CSF, I-TAC, Pentraxin-3, IL-8, IL-1β, IL-1RA, MIP-1α, IL-15, and IL-1α) were found to drive clustering of LC with Lung disease into three clusters: C1 (48.1%) has intermediate levels of cytokines and included a mix of LC and LD participants; C2 (38.1%) was skewed towards non-LD/non-LC participants and had elevated MIP-1α, IL-15, and IL-1α, and lower levels of T/NK cytokines; C3 (13.8%) was enriched for LC and LD and showed the reverse pattern, displaying an innate-skewed inflammatory profile marked by macrophage/neutrophil recruitment and reduced T-cell signals.

Gastrointestinal symptoms were reported only among those with LC, regardless of LD status in all three clusters. Pairwise logistic regression linked C3 with higher BMI and earlier symptom increases (~550 days post-symptom onset), whereas symptoms in C1 and C2 were lower and occurred later (~630 days post-symptom onset); neurological symptoms were also linked to C2. Spearman correlations highlighted LC variability even within comorbidity categories. Defining cytokine signatures of pre-existing comorbidities and complex immune dysregulation provides a more targeted approach to understanding the various manifestations of Long COVID.

61. Mechanical regulation induced functional heterogeneity in dendritic cells

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Dendritic cells play a pivotal role in orchestrating adaptive immunity, making them a promising target for immunotherapy. While autologous DC therapies have shown clinical potential, especially in cancer treatment, their efficacy remains limited due to weak effector functions in vivo and a lack of phenotypic heterogeneity from the ex vivo-generated dendritic cells. This study presents a novel approach to modulate DC phenotype and function by leveraging mechanical regulation in a three-dimensional microenvironment. Using a tunable hydrogel system with adjustable stiffness and viscoelasticity, stem cell-derived DCs exhibit heterogenous effector functions after being primed by distinct mechanical properties. Our findings show that matrix viscoelasticity significantly impacts DC phenotype, functions, and transcriptional profile. Analysis revealed that viscoelasticity-driven gene signatures map to tumor-infiltrating DC subsets and predict differential clinical outcomes in head and neck squamous cell carcinoma. Further, the elastic gene signature shows worse response to immune checkpoint blockade whereas the viscous gene signature shows better outcome. These results suggest that DCs can be mechanically primed by 3D hydrogels for ex vivo engineering of immunotherapies.

62. Focused Ultrasound Thermal Ablation and CD40 Agonism Yields Complete Responses Driven

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Background: Immunotherapies offer a promising therapeutic strategy for cancer, yet the lack of a ubiquitous response in breast cancer (BC) remains of particular concern given rising incidence among younger populations. Toward improved immunological control of BC, thermally ablative focused ultrasound (T-FUS) offers an incisionless, non-toxic intervention for tandem tumor debulking and immunomodulation. We here investigate the combination of T-FUS with costimulatory immunotherapy via CD40 agonism (αCD40) and probe mechanisms of T cell mediated immunity and complete response therein.

Methods: An αCD40 priming regimen was initiated one week prior to T-FUS in multiple murine BC models (E0771, 4T1, Brpkp110, EMT6). Subtotal T-FUS was performed with a custom ultrasound-guided FUS system. Tumor outgrowth and survival were monitored. Complete responders (CRs) underwent contralateral tumor rechallenge alongside age-matched, naïve controls. Flow cytometry was performed on tissue and serial blood samples.

Results: Tumors showed qualitative evidence of subtotal ablation one day post-T-FUS, marked by increased cleaved caspase-3, upregulated peri-ablative HSP70 expression, and significantly elevated and sustained intratumoral ATP liberation, a canonical marker of immunogenic cell death. T-FUS+αCD40 elicited superlative growth control and marked survival benefits compared to controls across BC models–yielding 33% CRs in the E0771 model. CRs' tumor draining lymph node bore markedly distinct T cell architecture in comparison to non-CRs, with significantly increased percentages of $CD4^+$ and $CD8^+$ T cells. Antibody depletion of $CD8^+$ and/or $CD4^+$ T cells revealed that protection was T cell-dependent. CRs exhibited full protection against tumor rechallenge, increased circulating

$CD44^+$,$CD62L^-$ $CD4^+$ T cell levels from week 1 to 3, and significantly elevated function ($IFN\gamma^+$,$TNF\alpha^+$) and CD40L expression on peripheral $CD4^+$ T cells.

Conclusions: T-FUS+αCD40 represents a novel breast immuno-oncology paradigm capable of conferring complete responses underscored by evidence of immunological memory. Ongoing studies include immunoPET imaging and blood biomarker assessments for forecasting responder/non-responder stratifications in the T-FUS+αCD40 setting.

63. Universal Allogeneic CART Product for Solid Tumors Targeting DSG2

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Background: Chimeric antigen receptor T (CART) cell therapies are a well-established therapy for hematological malignancies, with several FDA-approved therapies. However, autologous CART therapy (produced from the patient's own cells) encounters multiple obstacles, including high cost, long manufacturing timelines, manufacturing failure, and variability in product quality due to patient-specific factors. These challenges are more common in solid tumors. To overcome these limitations, we are developing an off-the-shelf, gene-edited allogeneic (donor-derived) CART cell therapy, which is designed for solid cancer treatment by targeting Desmoglein-2 (DSG2).

Methods: Using our established system, we isolated T cells from healthy donors and used lentivirus to express a DSG2-directed CAR. To design the allogenic CART, we are employing a cytosine base editor (CBE) to disrupt TCRαβ (to prevent graft-versus-host disease), β2-microglobulin (β2M), and CIITA (to eliminate MHC class I and II and halt immune rejection). CD54 and CD58 will also be knocked out to protect the allogenic CART from NK cell attack. CAR expression and gene editing efficiency are confirmed by flow cytometry and PCR. Functional assays include target cell killing, cytokine release, and proliferation. In vivo efficacy and safety will also be evaluated.

Results: Our preliminary data demonstrate that conventional DSG2-directed CART shows strong potency with a low risk profile in mouse models. Based on these findings, we are designing a universal CART using our previously designed DSG2 CART construct. Current efforts are focused on screening gRNAs for base editing and combinatorial editing.

Conclusions This work establishes the foundation for designing a universal, gene-edited CART therapy targeting DSG2. Once engineered and validated, this system may serve as a scalable, off-the-shelf strategy for treating solid tumors."

64. Multi-donor STORM atlas reveals lamina tethering and nanoscale chromatin reorganization encoding primary human macrophage polarization

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Macrophage polarization underlies host defense and tissue remodeling, yet how nanoscale chromatin architecture in primary human macrophages encodes these states and whether it establishes barriers to repolarization remain poorly understood. Here we apply stochastic optical reconstruction microscopy (STORM) to generate a comprehensive, multi-donor atlas of chromatin organization in human monocyte–derived macrophages polarized into distinct functional states: M0 (M-CSF–differentiated), M1 (classically activated; IFN-γ + LPS), and M2 (alternatively activated; IL-4 + IL-13). We perform super resolution imaging of DNA, histone, and critical epigenetic marks across multiple donors, systematically quantifying nuclear geometry, spatial chromatin distribution, including lamina-associated domain (LAD) occupancy and nanodomain size and spacing. Our results show that compared to M0 and M2 macrophages, M1 macrophages possess smaller, more elongated nuclei characterized by increased peripheral DNA localization and enhanced genome-wide compaction. These findings suggest a structural model where global chromatin compaction coexists with focal activation hubs, thereby reconciling compact bulk chromatin with the maintenance of inducible gene expression. Furthermore, we integrate Lamin B1 ChIP-seq from matched donors to map super-resolution features onto genome-

wide lamina proximity and accessibility. Additionally, through targeted disruption of the interaction between the cytoskeleton and nucleus envelop, as well as nucleus envelop and chromatin, we are testing whether lamina tethering represents the primary biophysical mechanism underlying barriers to macrophage repolarization between M1 and M2 states. Collectively, our framework connects chromatin–lamina interactions, and nanoscale chromatin organization to macrophage state control, with implications for reprogramming tumor-associated macrophages in immunotherapy.

65. Precise control of biomaterial chemical & physical parameters to regulate CAR-T cell persistence

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[abstract unpublished]

66. Nanobody-STING Agonist Conjugates to Improve Adoptive Cell Therapy for Solid Tumors

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Adoptive cell therapy (ACT) shows promise in treating solid tumors but remains limited by the immunosuppressive tumor microenvironment (TME), which restricts T cell infiltration and persistence. Stimulator of interferon genes (STING) agonists can remodel the TME, yet systemic delivery faces challenges of poor tumor accumulation and toxicity. We hypothesize that systemic delivery of a STING agonist, optimized through targeted nanobody design, can enhance ACT efficacy for solid tumors. To this end, we developed albumin-hitchhiking nanobody–STING agonists (AHNSA), which exploit albumin transport for improved tumor localization and controlled immune activation.

We evaluated AHNSA as an adjuvant to ACT in a TCR-transgenic T cell transfer model. First, we optimized dosing and timing of AHNSA with ACT to maximize antitumor activity. Next, we assessed adoptively transferred T cell phenotype, focusing on activation, proliferation, exhaustion, and cytotoxicity by flow cytometry and immunohistochemistry. Finally, we examined TME composition to define immune shifts induced by AHNSA.

Systemic AHNSA given after OTI T cell transfer conferred a significant survival advantage in mice bearing MC38-OVA tumors. Treatment increased infiltration of both innate and adoptively transferred T cells. Flow cytometry revealed that T cells in AHNSA-treated tumors were more activated, proliferative, and less exhausted. Bulk tumor analysis further showed enrichment of pro-inflammatory immune subsets (M1 macrophages, dendritic cells, CD8 T cells) and depletion of suppressive populations (myeloid-derived suppressor cells, M2 macrophages, regulatory T cells), indicating reversal of immunosuppression. Ongoing studies are testing this strategy in a fully immunocompetent murine CAR-T model.

Overall, this work establishes a novel immunotherapeutic approach for overcoming the barriers of ACT in solid tumors by coupling systemic STING agonism with nanobody-based targeted delivery.