Our understanding of cancer has and will continue to yield advancements in our ability to improve and extend patient's lives. Two major issues for cancer treatments, however, are resistance to drugs and lack of immune efficacy. In both of these cases, complex signaling networks are perturbed and even share some molecular logic. Quantitative models are an important component in identifying, communicating, and targeting this dysregulation. However, the physical relevance of any model is ensured only through careful pairing with the appropriate experimental evidence.

We intend to utilize close integration of signaling measurement, rigorous modeling, and novel interventions to study and overcome each of these issues. Each of the three aims described here will employ these techniques in combination (Fig 1). In the first aim, we will focus on tumor cell-intrinsic targeted therapy resistance in order to understand how bypass pathway activation leads to resistance. Doing so will identify targetable, essential kinase signaling activation events. In the second aim, we will use data-driven analytical methods to further understand TAM (Tyro3, AXL, MerTK) receptor tyrosine kinase (RTK) function and rationally design innate immune-targeted agents. We will integrate both of these areas in the third aim, and pioneer approaches for measuring and manipulating immune-tumor cell communication to identify more effective therapeutic combinations targeting both. In the course of these studies, we will develop effective strategies for precise care taking into account signaling network dysregulation.

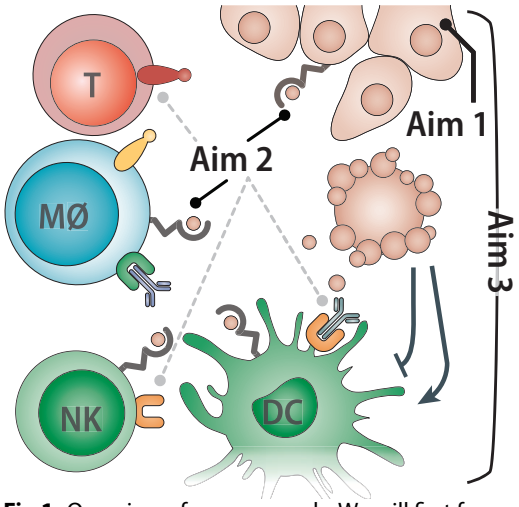

Fig 1: Overview of our approach. We will first focus on tumor cell-intrinsic targeted therapy resistance, studying how activation of non-targeted RTKs (bypass activation) leads to resistance (Aim 1). In Aim 2, we will apply data-driven analytical methods to elucidate TAM RTK function and apply these approaches to rationally design innate immune-targeted agents. Parallels between receptor families will make our methodological developments valuable for studying other innate immune receptors. We will pioneer approaches for measuring and manipulating immune-tumor cell communication in Aim 3 to identify more effective therapeutic combinations targeting both. Each of these areas will take advantage of thorough modeling and experimental integration.

My experience with quantitative biochemical methods, multivariate statistics, applied machine learning, and a deep understanding of the underlying biology provide me with a unique intersection of skills at this interface. Many of the systems-level techniques being applied currently to study tumor cell-intrinsic resistance will be vital to maximizing the effectiveness of immune targeted therapies. My prior research related to TAM RTKs lies at the interface of cancer biology and innate immune regulation, providing me with a unique background to link these areas[1,2]. We will focus on cancer therapeutic resistance and immune escape, considering inflammatory diseases such as lupus, viral infection, rheumatoid arthritis, and endometriosis[3–5], and simultaneously pursue collaborations for application of our approaches in translational settings and expansion into new biological areas of investigation.

Aim 1: Identifying Shared Features Among Resistance Mechanisms to Help Predict Individual Patients' Effective Combination Therapies

Targeted therapies extend many cancer patient's lives, but are limited in efficacy to a subset of patients and by the development of resistance. Enormous efforts undertaken to identify mechanisms of resistance have uncovered numerous changes involving gene expression, post-translational regulation, and even tumor-extrinsic factors such as host-derived growth factors[6,7]. Combination therapy can effectively combat resistance, but requires accurate identification of the relevant resistance mechanism. Precision therapy must account for many genetic and non-genetic intrinsic and adaptive resistance mechanisms to accurately select these combinations.

Rather than focus on single molecular changes causing resistance, we are studying sets of these changes to reveal the essential commonalities. Methods studying the signaling network changes driving resistance to date have largely focused on two approaches: (1) paired molecular and response measurements across large panels of cell lines[8,9], or (2) screening-based platforms in which a large panel of expression changes are assessed for effect on resistance[7,10,11]. These are complementary and informative but limited in the information provided in key aspects. In the former case, one is limited to the variation within the cell line panel, with the ultimate resistance mechanism often unknown. Further, widespread genetic variation between cell lines serves as "noise" diluting out the "signal" of exactly which signaling

changes lead to resistance, and may not be representative of resistance derived from sources such as the tumor microenvironment. In the latter case, sparse accounting for the molecular changes present with each intervention limits the commonalities that can be identified. Pinpointing the necessary and sufficient signaling changes for resistance mechanisms is essential for a clearer picture of what to measure and target in individual patient's tumors. For example, if bypass resistance to EGFR inhibitors in lung adenocarcinoma mediated by cMET, AXL, and FGFR1 all rely on Erk *or* JNK activation mediated by Grb2 *or* CrkL, then we can use this logic to identify precise treatment combinations. One might, with this knowledge, target AXL if activated cMET and FGFR1 are not present, or CrkL if Grb2 is not active. Realizing precision medicine in this fashion requires identification both of the essential molecular events for resistance and mapping between the various signaling layers.

We are currently mapping this logic as part of my NIH Director's Early Independence Award to understand resistance to targeted kinase inhibition mediated by non-targeted receptor tyrosine kinases (RTKs)—so-called bypass resistance (Fig 2). A central method for this work is to use cells sensitive to an RTK inhibitor and then rescue them to varying extents with a panel of growth factors, cytokines and/or transient expression of selected genes. We then quantify the apoptosis/proliferative response of the cells in the presence of the inhibitor in each case, paired with multiplexed signaling measurement. Using multivariate modeling, we identify the molecular features that predict resistance, then experimentally verify their involvement. This exemplifies the benefit of close integration between the experimental and modeling efforts—through matched genetic backgrounds with very little time for subclonal selection, this approach provides optimal measurements for the modeling question at hand.

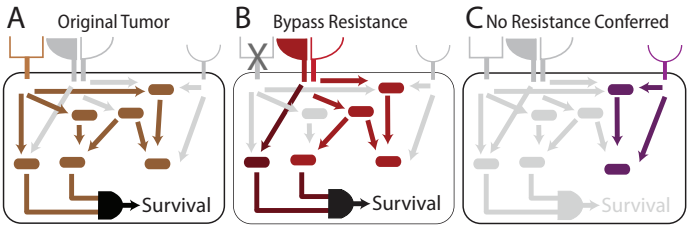

Fig 2: (A) In RTK-driven tumors, signals are transduced from the receptor to various kinases. (B) Upon blocking the original cancer driver, resistance can be conferred by an untargeted receptor[12]. (C) Some receptors, however, do not provide essential resistance signals. By identifying similarities and differences of signaling from each receptor, we will be able to identify measurements pinpointing the relevant receptor causing resistance.

This framework has already led us to identify that pathways beyond just canonical markers such as Erk/Akt are important for identifying whether an RTK can make cells resistant to EGFR- and HER2-targeted therapies. Namely, JNK activation is essential for predicting resistance, and modulating JNK activity in concert with other pathways can influence resistance development[13]. Having published this initial work as a proof of concept, we are now applying this to identify drug combinations and study tumor heterogeneity. Lung cancer cells display dynamic, heterogeneous activation of Erk and JNK, and so we are mapping the multi-pathway single cell variation in activation as a potential mechanism of tumor persistence and adaptive resistance. In collaboration with Eric Haura (Moffitt Cancer Center), we are also testing whether receptor-proximal measurements can identify which RTK is driving disease, and in turn can predict optimal treatment combinations for individual xenograft tumors.

Identifying commonalities among the many expression and signaling changes that cause cells to become resistant is a critical compliment to the functional genetics studies that have now globally mapped these changes[7,10,11]. Using this approach, we will be able to determine whether cells reactivate the same downstream kinases or alternatively rely on fundamental changes in pathway activation dependency for a wider panel of molecular network changes driving resistance. This map will enable us to identify effective combination therapies for individual tumors given measurements for which kinases are active.

Aim 2: Systems Approaches for Rationally Designing Innate Immune Therapies

Through my previous work involving the TAM RTK family, I have developed seamless capabilities spanning tumor cell and immune systems biology. These receptors are implicated in resistance to targeted therapies and metastasis via tumor cell-intrinsic effects, while more recent evidence has implicated the same receptors expressed on immune cells as a potentially effective therapeutic targets in many cancers (Fig 3). Outside of cancer, these receptors have been implicated in a number of diseases involving immunological dysregulation including lupus, rheumatoid arthritis, endometriosis, viral infection, and asthma[14–17]. Rationally targeting these receptors, and even understanding how existing therapies function, has been limited by poor understanding of how the receptors are activated.

As efferocytosis receptors, a principal function of TAMs is to drive phagocytosis of phosphatidylserine-presenting

extracellular debris via their ligands. Consequently, studying the receptors requires taking into account receptor function, ligand engagement, and the role of lipid vesicles. Work from my lab recently proposed, using a combination of modeling and experimental validation, that spatially-defined ligand presentation is vital to activation of these receptors[2]. Spatial patterning underlies nearly all signaling processes. However, developing models incorporating spatial signaling aspects has been hampered both by difficulties in computationally accounting for these factors as well as experimentally manipulating them during model training and validation[18–21]. This, therefore, represents a rich area for both modeling and experimental methodological development.

To expand upon this initial model, my lab has been and will continue modeling the activation and subsequent phenotypic effects of TAM receptors within tumor and immune cells[1,2]. Currently, we are quantitating the pattern of expression and measuring kinetic binding parameters necessary to simulate activation of the TAM receptors across many immune cell types. To rapidly develop models of receptor activation, we are rigorously accounting for the uncertainty in our models using Bayesian methods. We are taking advantage of our deep experimental and computational integration by utilizing, for example, a panel of receptor fragments, each with distinct binding properties. These represent unique TAM-targeted agents with therapeutic potential and specific inhibition profiles toward certain cell populations or activation mechanisms. At the same time, their specific effects will provide novel interventions to help deconvolve the pleiotropic roles of these receptors *in vivo*.

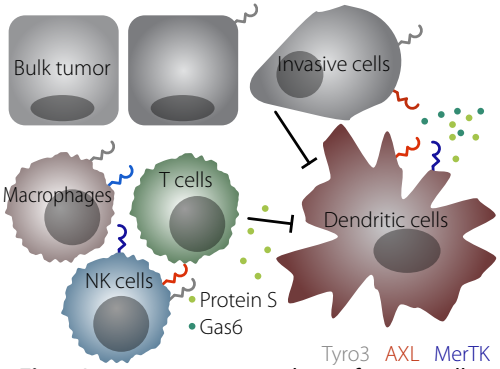

Fig 3: In many cancers, a subset of tumor cells overexpress AXL, making them invasive and resistant to therapy. TAM receptor activation within dendritic cells potently inhibits the innate immune response[22]. T cell release of ProS further dampens the immune response[23]. Activation of TAMs inhibits NK cell-mediated lysis[24]. Each of these cell populations express distinct and dynamic combinations of TAM receptor, likely modulating functional changes in microenvironmental response[24–27].

More broadly, maximizing the potential of immune-targeted therapies will require an improved, multi-scale understanding of tumor-immune interaction from the molecular level to that of cell-cell interactions. Like TAMs, many receptors such as the Fc family and complement receptor are poorly understood both in their proximal activation and their downstream signaling effects, have simultaneous roles in signaling and trafficking, are activated through clustering as opposed to strictly receptor-ligand interaction, and derive specificity through the combination of activated species. Thus, TAM receptors represent a valuable prototype for studying immune receptor function on a systems level more generally. Indeed, we are beginning to take a similar approach through a collaboration with Falk Nimmerjahn (U. Erlangen-Nürnberg) targeting IgG effector function across cell populations using multivariate models of multivalent FcγR-IgG interaction.

Aim 3: Measuring and Integrating Tumor & Immune Response to Optimally Target Both

Realizing the maximal benefit of immune-targeted therapies will come through a better understanding of the ongoing communication between tissue microenvironments and the resident immune cells. In cancer, this will be derived in part by integrating how targeted therapies, immunotherapies, and cytotoxic therapy operate coordinately[28]. The breakdown of these tissue-immune interactions also leads to a variety of diseases including lupus and endometriosis[16,29].

Targeted therapies have the potential to operate almost as an *in situ* vaccine by driving tumor cell death[30]. However, these therapies are often cytostatic, or even upon maximal response induce apoptosis, a largely immunosuppressive process. Multivariate signaling models have been extensively used to study tumor cell response to various cues, almost exclusively using measures of viability or apoptosis as a response[31,32]. In contrast, we will build models with quantitation of apoptosis, non-apoptotic response (such as necroptosis), and damage-associate molecular patterns, examining combinations of cytotoxic agents and targeted inhibitors[33]. Models of the signaling pathway contributors to immunogenic cell death will allow targeted therapy combinations to be designed to promote an effective follow-on immune response.

A critical communication junction between tissue homeostasis and immunosurveillance is antigen trafficking (Fig 4). Efferocytosis, the phagocytosis and processing of debris from apoptotic cells, is on one hand an important mechanism for antigen trafficking within the innate immune system but simultaneously strongly immunosuppressive[22,34]. This suppression is critical to sustaining self-tolerance—efferocytosis defects are linked to immunological disorders such as

lupus—but also limits the desirable immunogenicity of cytotoxic therapies in cancer[16]. Many efferocytosis receptors, including MFGE8, TAM RTKs, and TIM receptors, that direct this process, and other signals (such as lipid oxidation) within extracellular debris can also lead to uptake by antigen-presenting and other cells[35,36]. Non-phagocytic cell types also participate in the homeostatic turnover of debris[37,38]. The unique challenges in understanding the regulatory factors during efferocytosis make it an especially interesting area for application of engineering methods. For example, biophysical properties such as size likely influence trafficking of debris among distinct cell types. Competition between cell types is intrinsically multivariate, and so will benefit from analysis methods to understand the critical regulatory factors modulating trafficking. Through a combination of measurement and manipulation, we plan to both understand the critical factors and rationally modulate debris trafficking. Using labeled, artificial debris in the form of lipid vesicles we can track the trafficking of this debris, and how it changes in distinct microenvironments. Analytical techniques such as flux balance analysis will be critical to interpreting the pleiotropic changes that occur in debris trafficking with any intervention. Mapping the factors that influence the destination of apoptotic debris has important consequences for multiple areas, from vaccine design to promoting an immune response to targeted cancer agents.

Longer-term, I envision our approaches examining bypass signaling with tumor cell-intrinsic resistance (Aim 1) will become critical to ensuring broad immunotherapy efficacy. The first immune-targeted therapies are showing remarkable success, and combinations are in mid-stage clinical studies, yet tumor-specific variation in mechanism will present a similar challenge with immune-targeted agents as it has for targeted therapy resistance[39,40]. With a plethora of targets including PD1, TIM3, CTLA4, CD40, LAG3, and OX40, we will need new measurements and models to effectively identify the relevant combinations for particular tumors[41]. The timing and combination of agents will play an important role in maximizing the breadth of patients who benefit. My lab will be uniquely positioned to address this problem with systems methods.

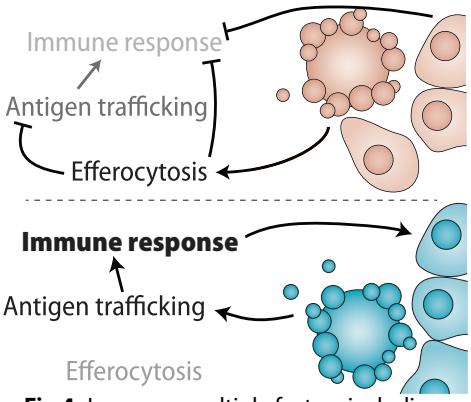

Fig 4: In cancer, multiple factors including efferocytosis and direct signaling lead to an immunosuppressive environment. In contrast, diseases with autoimmune contributions are linked to aberrant cell death and/or cellular clearance, leading to positive feedback disrupting self-tolerance. Through this similarity, studying the signaling and trafficking effects of cellular debris has important implications for both cancer and these diseases.

Lab Composition & Connections The work here will develop models of cancer and immune signaling dysregulation, and how these mechanisms vary across individuals, by closely integrating novel experimental measurements and computational modeling. These efforts will fundamentally impact the treatment of cancer and will extend into other disease areas. Due to the breadth of disciplines and expertise required to develop and apply these approaches, I envision my lab will include students and postdoctoral associates from varied backgrounds, including chemical and biological engineers, biologists and data scientists. I also look forward to fruitful collaborations will extend these research initiatives within and outside the department, through such organizations as the Chemical and Biomolecular Engineering Department, Parker Institute for Cancer Immunotherapy Center, and Department of Microbiology, Immunology, and Molecular Genetics.

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