CHEMICAL ENGINEERING

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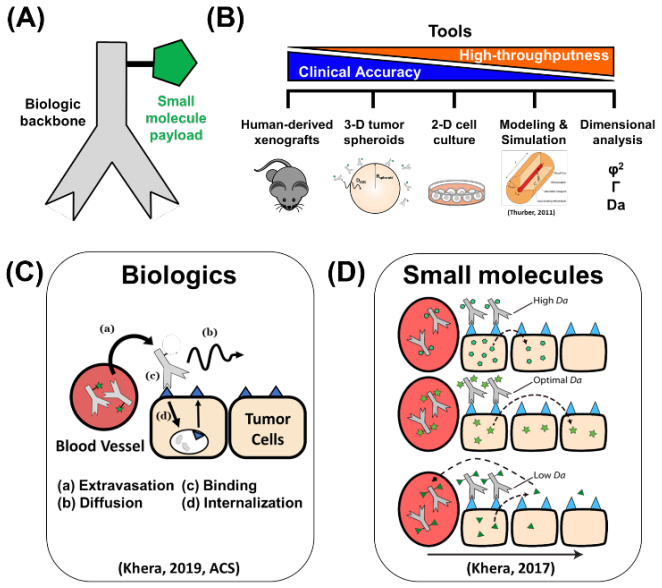
Redefining the Rules: Engineering Tumor Distribution of Therapeutics Beyond Rule-of-Five

ABSTRACT: Drug design and discovery is a continuous optimization problem that has stumped chemists for decades. Prior to advances in machine learning that establishes "desirable" drug properties (Winter, 2019), the empirical but iconic Lipinski's Rule of Five (Ro5) was proposed. Originally a "rule-of-thumb" for the identification of drugs likely to be absorbed orally, Ro5 soon became overemphasized as the absolute criteria for drug design. Identification of several successful non-Ro5 drugs has initiated a shift from Ro5 overuse, but the fundamental question remains – how do we establish guidelines for successful drug design beyond Ro5 (bRo5)? Using antibody-drug conjugates (ADC) as a model system, I will highlight how mechanistic analysis has turned the field of ADC design upside-down, with design approaches that directly contradict the dogma of the field (Beck 2017) (e.g. TrodelvyTM contains large doses of low potency payloads instead of small doses of newer ultrapotent payloads) driving accelerated ADC clinical success, with 3 FDA approvals within the past few months compared to the total 8 in the last 3 decades. Given their unique and complex composition (Fig. A), ADCs embody diverse bRo5 properties, and lessons learned from mechanistic ADC drug design can be generalized to other therapeutic indications.



bRo5 drugs dominate the field of cancer therapeutics, partially from being discovered prior to establishment of Ro5, and partially due to > 80% of cancer targets being "undruggable" by Ro5 small molecules (Verdine, 2007). bRo5 cancer therapeutics can largely be classified as small molecules and biologics. Each varies widely in physicochemical properties, mechanisms of action, and in vivo behavior, precluding empirical one-size-fits-all guidelines. However, revisiting the analysis that generated Ro5 (Lipinski, 2001) reveals that before being veered off-course by empiricism and a hyperfocus on oral delivery, Ro5 origins were based on fundamentals of drug pharmacokinetics, highlighting the potential of a mechanistic approach to bRo5 drug design. Mechanistically, cancer drugs can be categorized based on four fundamental rate-limiting processes: blood flow (Class I), extravasation (Class II), diffusion (Class III), and elimination (Class IV) (Thurber, 2011). Evaluation of cancer drugs has historically involved detailed analysis of drug uptake, but the intratumoral distribution is still not well-understood. Using antibody-drug conjugates as a platform to study both bRo5 small molecules and biologics (Fig. A), I employed dimensional analysis, in silico, in vitro, in vivo tools (Fig. B) to quantify intratumoral drug disposition as a function of drug diffusion and drug elimination.

Antibody-based biologics often display perivascular intratumoral distribution, accumulating only in cells close to blood vessels, leaving distant cell untreated. This well-known issue arises from rapid immobilization of the antibody onto cell surface receptors before it can diffuse very far (Fig. C), yet the field has a limited understanding on how to overcome this issue. Using a panel of single-domain antibodies we show cellular internalization is the key parameter influencing antibody intratumoral distribution, with faster internalization driving worse distribution and therefore worse efficacy (Nessler, Khera, 2019), a direct contradiction to existing ADC design recommendations. However, this again is not a one-size fits all rule, and show that the dimensionless Thiele modulus, φ2 (diffusion & binding rate/internalization rate) can be used to rapidly evaluate the impact of ADC internalization on distribution. We also show how this theoretic analysis can be used for refining clinical recommended phase 2 dosing (RP2D) of monoclonal antibodies.

In the second part of this work, I developed a comprehensive "systems approach" to quantify intratumoral distribution of a diverse panel of bRo5 small molecules, evaluated via bystander effects of antibody-drug conjugates, wherein intracellular ADC degradation releases payload that can diffuse out of targeted cells and leak into adjacent untargeted cells. I first developed a fully predictive partial differential equation Krogh cylinder model that predicts subtle differences in small molecule distribution based solely on the molecular weight, lipophilicity, and charge of the molecule. The dimensionless Damköhler number (extracellular diffusion rate/passive cellular uptake rate) predicts tumoral distribution of small molecules (Fig. D), with an optimal balance between diffusion and cellular uptake at Da ~ 3 (Khera, 2017). Next, I developed a high-throughput framework to experimentally track payload distribution in 3-D spheroids and in vivo tumors by re-purposing veteran pharmacodynamic markers as a proxy for payload tracking. This work shows that lethal payload concentration penetrates at least twice as far as the ADC, demonstrating for the first time direct cellular-resolution evidence of bystander payload killing, a phenomenon previously observed only empirically. The final, and ongoing, phase of this work is to integrate the high-throughput experimental and computational frameworks with the Damköhler analysis to develop a workflow to estimate the "bystander potential"/intratumoral disposition of a panel of bRo5 payloads. Correlation of outcomes from this multi-system platform will objectively validating Da as a tool for rapid prediction of small molecule disposition.

Together, these works present a holistic and mechanistic approach to establishing drug design guidelines for both small molecule and biologic cancer therapeutics not administered orally. While the conclusions presented here may appear intuitive in hindsight, it is crucial to remember that they remained elusive for nearly four decades of ADC development until revealed by mechanistic analysis. More importantly, it validates that in vivo behavior of drugs is not an independent sum of their individual physicochemical properties. Rather, these properties work synergistically to influence drug behavior, and drug design guidelines must be formulated with keeping this interdependence in mind. Dimensionless analysis is a valuable chemical engineering tool that can simply yet effectively capture this interdependence, and rapidly predict the behavior of any drug, catalyzing a shift beyond Ro5 to rational guided drug design.

BIOGRAPHY: Eshita Khera is a Ph.D. candidate at the University of Michigan, studying the design of imaging agents and cancer therapeutics under the mentorship of Professor Greg Thurber. She has earned a B.Eng. in Biotechnology from PES Institute of Technology, India and an M.S. in Biomedical Engineering from the University of Michigan. As a master's student, Eshita conducted research in the Wen lab on engineering virus-like particle vaccines against Ebolavirus and is listed as a co-inventor on a patent for the same. In 2019, Eshita completed a 12-week internship in the Pharmacometrics group at Novartis (Cambridge, MA) on mechanistic modeling of receptor occupancy for recommended phase 2 dosing of oncology antibodies. Outside research, Eshita is an active campus leader and has founded/co-founded several initiatives, including a science communication workshop & seminar series and a first-of-its-kind student-led ChE undergraduate research course. She is a member of the American Chemical Society and the Tau Beta Pi Engineering Honor Society. In her free time, Eshita enjoys trivia nights with friends, yoga, badminton, painting/sketching, reading, and (very) amateur astrophysics.

LECTURE 1:00 - 2:00 Zoom
Networking Hour on Zoom Following

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