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Basic/Translational Science/Team Science

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A Mouse Model of APOE Genotype in Chemotherapy Related Cognitive Impairment

Tamar Demby[1], G. William Rebeck[1], Christopher Albanese[1], Olga C. Rodriguez[1], Yichien Lee[1] and Jeanne Mandelblatt[1]
[1]Georgetown - Howard Universities

OBJECTIVES/SPECIFIC AIMS: Chemotherapy-related cognitive impairment (CRCI) affects 15-35% of breast cancer survivors and constitutes a significant challenge for survivor quality of life. Among older breast cancer survivors who received chemotherapy treatment, carriers of at least one ε4 allele of the APOE gene, which encodes apolipoprotein E, are at higher risk for developing CRCI than non-carriers. APOE4 is well characterized as the strongest genetic risk factor for Alzheimer's disease, but how it contributes to CRCI is not yet understood, and no animal models of APOE genotype and CRCI have yet been established. To better understand how APOE4 acts as a risk factor for CRCI, we used APOE targeted replacement (TR) mice to develop a model of its effects on cognition following treatment with doxorubicin, a chemotherapy drug commonly used in breast cancer treatment. METHODS/STUDY POPULATION: Twelve-to-thirteen month old APOE3 and APOE4 targeted replacement mice expressing human APOE3 or human APOE4 under control of the endogenous murine promoter were treated with 10 mg/kg doxorubicin or equivolume saline given via two IP injections spaced one week apart. One week post-treatment, mice were tested using Open Field and Elevated Zero apparatuses to assess baseline locomotive activity and anxiety and exploratory behaviors. Five weeks post-treatment, mice were assessed using the Barnes Maze over four days of training trials and one 72 hour memory probe. RESULTS/ANTICIPATED RESULTS: We found no differences in Open Field and Elevated Zero behavior, indicating limited influence of doxorubicin treatment on locomotive and anxiety behaviors in both genotypes. During Barnes Maze training, APOE4 mice treated with doxorubicin showed increased latency compared to untreated APOE4 mice as well as treated and untreated APOE3 mice, indicating deficiencies in spatial learning. In APOE3 mice, no differences in performance were seen between doxorubicin-treated and untreated mice ($n = 15\text{-}16$/group, $p < .0001$). DISCUSSION/SIGNIFICANCE OF IMPACT: These results indicate that APOE4 targeted replacement mice have specific cognitive vulnerabilities to doxorubicin treatment that can be reliably detected using the Barnes Maze assessment. Future directions include experiments to determine how other chemotherapy drugs or drug combinations impact cognition of APOE4 mice. Ultimately this model may be used to assess preventive measures or therapies for CRCI in the vulnerable APOE4 carrier population with the ability to validate cognitive impacts of these interventions.

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Advancing Glioblastoma (GBM) drug regimen development to support combination therapy through integrated PKPD modeling and simulation-based predictions

Saugat Adhikari[1], Harlan E. Shannon[1], Karen E. Pollok[1] and Robert E. Stratford[1]
[1]Indiana University School of Medicine

OBJECTIVES/SPECIFIC AIMS: Despite advancements in therapies, such as surgery, irradiation (IR) and chemotherapy, outcome for patients suffering from glioblastoma remains fatal; the median survival rate is only about 15 months. Even with novel therapeutic targets, networks and signaling pathways being discovered, monotherapy with such agents targeting such pathways has been disappointing in clinical trials. Poor prognosis for GBM can be attributed to several factors, including failure of drugs to cross the blood-brain-barrier (BBB), tumor heterogeneity, metastasis and angiogenesis. Development of tumor resistance, particularly to temozolomide (TMZ), creates a substantial clinical challenge.The primary focus of our work is to rationally develop novel combination therapies and dose regimens that mitigate resistance development. Specifically, our aim is to combine TMZ with small molecule inhibitors that are either currently in clinical trials or are approved drugs for other cancer types, and which target the disease at various resistance signaling pathways that are induced in response to TMZ monotherapy. METHODS/STUDY POPULATION: To accomplish this objective, an integrated PKPD modeling approach is used. The approach is largely based on the work of Cardilin, et al, 2018. A PK model for each drug is first defined. This is subsequently linked to a PD model description of tumor growth dynamics in the presence of a single drug or combinations of drugs. A key outcome of these combined PKPD models are tumor static concentration (TSC) curves of dual or triple combination drug regimens that identify combination drug exposures predicted to arrest tumor growth. This approach has been applied to TMZ in combination with abemaciclib (a dual CDK4/6 small molecule inhibitor) based on data from a published study evaluating abemaciclib efficacy in combination with TMZ in a glioblastoma xenograft model (Raub, et al, 2015). RESULTS/ANTICIPATED RESULTS: A PKPD model was developed to predict tumor growth kinetics for TMZ and abemaciclib monotherapy, as well as combination therapy. Population PK models in immune deficient NSG mice for temozolomide and abemaciclib were developed based on data obtained from original and published studies. Subsequently, the PK model was linked to tumor volume data obtained from U87-MG GBM subcutaneous xenografts, again using both original data as well as data from the Raub, et al, 2015 study. Model parameters quantifying tumor volume dynamics were precisely estimated (coefficient of variation < 30%). The developed PKPD model was used to calculate plasma concentrations of TMZ and abemaciclib that would arrest tumor growth, as well as combinations of concentrations of the two drugs that would accomplish the same endpoint. This so-called TSC curve for the TMZ and abemaciclib combination pair evidenced

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an additive effect of the two agents when administered together. These results will be presented. In addition, results from on-going PKPD studies of TMZ in combination with two other small molecule inhibitors, RG7388, an MDM2 inhibitor, and GDC0068, an AKT inhibitor, will also be presented. DISCUSSION/SIGNIFICANCE OF IMPACT: Our long-term goals are to further elucidate SOC-induced responses in GBM and establish combination treatment regimens that are safe and significantly improve therapeutic efficacy. Collectively, our studies will broadly influence chemotherapy of GBM by establishing a process to rationally design combination approaches that mitigate resistance development. These studies will ultimately provide opportunities to study other targeted agents tailored to individual molecular signatures of GBM, as well as other tumor types.

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An Evaluation of Machine Learning and Traditional Statistical Methods for Discovery in Large-Scale Translational Data

Megan C Hollister[1] and Jeffrey D. Blume[1]
[1]Vanderbilt University Medical Center

OBJECTIVES/SPECIFIC AIMS: To examine and compare the claims in Bzdok, Altman, and Brzywinski under a broader set of conditions by using unbiased methods of comparison. To explore how to accurately use various machine learning and traditional statistical methods in large-scale translational research by estimating their accuracy statistics. Then we will identify the methods with the best performance characteristics. METHODS/STUDY POPULATION: We conducted a simulation study with a microarray of gene expression data. We maintained the original structure proposed by Bzdok, Altman, and Brzywinski. The structure for gene expression data includes a total of 40 genes from 20 people, in which 10 people are phenotype positive and 10 are phenotype negative. In order to find a statistical difference 25% of the genes were set to be dysregulated across phenotype. This dysregulation forced the positive and negative phenotypes to have different mean population expressions. Additional variance was included to simulate genetic variation across the population. We also allowed for within person correlation across genes, which was not done in the original simulations. The following methods were used to determine the number of dysregulated genes in simulated data set: unadjusted p-values, Benjamini-Hochberg adjusted p-values, Bonferroni adjusted p-values, random forest importance levels, neural net prediction weights, and second-generation p-values. RESULTS/ANTICIPATED RESULTS: Results vary depending on whether a pre-specified significance level is used or the top 10 ranked values are taken. When all methods are given the same prior information of 10 dysregulated genes, the Benjamini-Hochberg adjusted p-values and the second-generation p-values generally outperform all other methods. We were not able to reproduce or validate the finding that random forest importance levels via a machine learning algorithm outperform classical methods. Almost uniformly, the machine learning methods did not yield improved accuracy statistics and they depend heavily on the a priori chosen number of dysregulated genes. DISCUSSION/SIGNIFICANCE OF IMPACT: In this context, machine learning methods do not outperform standard methods. Because of this and their additional complexity, machine learning approaches would not be preferable. Of all the approaches the second-generation p-value appears to offer significant benefit for the cost of a priori defining a region of trivially null effect sizes. The choice of an analysis method for large-scale translational data is critical to the success of any statistical investigation, and our simulations clearly highlight the various trade-offs among the available methods.

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An Injectable Sulfonated Reversible Thermal Gel for Controlled and Localized Delivery of Vascular Endothelial Growth Factor to Promote Cardiac Protection After a Myocardial Infarction

Adam J Rocker[1], David Lee[1], Maria Cavasin[1] and Daewon Park[1]
[1]University of Colorado Denver

OBJECTIVES/SPECIFIC AIMS: This study aims to evaluate an injectable sulfonated reserve thermal gel (SPSHU-PNIPAM) for angiogenic growth factor delivery by examining the vascularization and cardioprotective properties of the polymer system. This study could lead to clinical translation by moving into larger animal studies and eventually clinical trials. The success of this study was determined by analyzing the results of echocardiography data on cardiac function (ejection fraction, fractional shortening, and left ventricle inner diameter) and assessment of histological staining on cardiac tissue (fibrotic tissue formation, infarct size, wall thinning, blood vessel cell counts, and vessel size quantification) after MI. Five groups were compared for this study: saline, VEGF, SPSHU-PNIPAM, SPSHU-PNIPAM loaded with VEGF, and no injection (sham). Significant statistical differences between control groups and polymer injection groups, when $p < 0.05$, indicates successful outcomes from this study. METHODS/STUDY POPULATION: SPSHU-PNIPAM Polymer Synthesis: SPSHU-PNIPAM was synthesized as previously described. Briefly, PSHU was synthesized with N-BOC serinol, urea, and HDI at 90 °C for 7 days. PSHU was deprotected in DCM and TFA at room temperature for 45 min. PNIPAM was conjugated to the deprotected PSHU using EDC and NHS at room temperature for 24 h. PSHU-PNIPAM was sulfonated with 1,3-propanesultone and potassium tert-butoxide at 60 °C for 3 days. Surgical Procedure: Male C57BL/6 mice weighing 24-28 g were anaesthetized using isoflurane and artificial ventilation provided. A small left thoracotomy incision was made at the left fourth intercostal space to expose the heart, and the proximal left anterior descending coronary artery was ligated for 45 min. The coronary artery was then released and 30 µl injections of saline, SPSHU-PNIPAM (1% w/v), bolus VEGF (200 ng), or SPSHU-PNIPAM + VEGF (1%, 200 ng) were injected intramyocardially at the infarcted site and the incision closed. Echocardiography and Histological Staining: Standard serial transthoracic echocardiography was performed while simultaneously recording ECG to assess cardiac morphology and left ventricular function. Immunohistochemistry and histology staining procedures were used to identify: fibrotic tissue formation, infarct size, wall thinning, blood vessel cell counts, and vessel size quantification. These were performed according to manufacturer instructions or by previously published criteria. Statistical Analysis: Two-tailed t-test assuming unequal variances was used to determine significant differences between two groups. Analysis of variance (ANOVA) was used to determine significant differences between three or more groups followed by Tukey-Kramer to determine significant differences between two groups as appropriate. Statistical significance was considered when $p < 0.05$. References: Lee, D. J., Rocker, A. J., Bardill, J. R., Shandas, R. and Park, D. (2018), A sulfonated reversible thermal gel for the spatiotemporal control of VEGF delivery to promote therapeutic angiogenesis. J Biomed Mater Res. doi:10.1002/jbm.a.36496. RESULTS/ANTICIPATED RESULTS: Echocardiography results: Ejection fraction improved for

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