KPCRTF State Funded Projects Reporting Template

University of Kentucky – Project #4
Chemotherapy- induced cognition impairment – Mechanisms and Prevention
Program Director: Daret St. Clair

Reporting Period: _____April - June 2021_____

Below please provide a brief summary of the status of the Project listed as well as for each Aim listed below. Include any barriers, how and if they were overcome, and successes achieved.

During the final reporting period of April to June 2021, our ability to meet our goals continued to improve because we have full laboratory research capacity. We have made excellent progress toward our ultimate goal of identifying intervention that will prevent cognitive impairment in children with ALL. We have met our goal of recruiting 20 patients to the study. However, not every patient can provide a blood sample at all time points scheduled. Thus, we plan to continue to recruit additional patients to complete the study. This study is very important so we want to make sure that we have a sufficient sample size to rigorously analyze the results. Although the PTRCF support has ended, we will continue to recruit ALL patients to the study. Our results obtained thus far are consistent with our hypothesis that redox stress leads to the release of extracellular vesicles (EVs) into circulation, as EVs can cross the blood- brain barrier. EVs then activate immune cells that produce pro-inflammatory cytokines such as TNF-α, which are neuropathogenic.

Aim 1: Evaluate EVs for their effect on human immune cells to determine the mechanistic links between circulating EVs and therapy-induced immune activation

We have identified methods for isolating EVs that not only requires a minimum amount of blood but is also highly reproducible. We compared three isolation methods: two are well-established methods of EVs isolation and the third is a newly developed method that uses size- exclusion chromatography in a 96- wells format SmartSEC HT EVs Isolation System. The results indicate that the simple ExoQuick method, which is one of the two well-established methods for isolating EVs, provides the highest yield of EVs proteins. However, we have previously shown that the ExoQuick method significantly contaminates isolated EVs with serum proteins. ExoQuick Ultra adds a chromatography step that provides clean EVs with the ability to activate the immune system, but the yield is drastically reduced. The SmartSEC system also employs the chromatography step used in the ExoQuick Ultra and it provides the greatest yield. Thus, we have chosen to use the ExoQuick Ultra method for the isolation of EVs from established ALL cell lines in culture and the SmartSEC method for isolation of EVs from human serum.

We have optimized an assay using macrophages and bone marrow derived macrophage (BMDM) treated with EVs. Our results from ALL cell lines and serum from children with ALL suggest that EVs obtained from ALL samples can activate immune cells to produce pro-inflammatory cytokines such as TNF-α and IL-6. Once we have obtained the number of patients needed, we will repeat these experiments to confirm the initial results.

Aim 2: Determine the effect of MESNA on immune activation and neuronal injury to investigate the immune-mediated mechanism of CICI

A major goal of this study is to test whether antioxidants can mitigate against cancer therapy-induced, TNF α -mediated neuronal injury. We first tested whether addition of an FDA- approved antioxidant would reduce the therapeutic effect of the prototypical anticancer drug methotrexate (MTX) that is used in all phases of ALL therapy. We tested the effect of MESNA (2-mercaptoethane sulfonate), a cell impermeable antioxidant, on lymphoma cell response to MTX. The results indicate that the inclusion of MESNA does not reduce the effectiveness of chemotherapy to kill ALL cells. We also tested EVs released from ALL cells to determine the effect of MESNA in activating cytokine from immune cells. The results indicate that EVs isolated from human B lymphoma, Reh, cells, can activate macrophages to produce the neurotoxic cytokine TNF-α and that addition of MESNA reduced the TNF- α production by macrophages.

Aim 3: Characterize EVs isolated from children before and after completion of chemotherapy to gain insights into mechanisms of brain injury after systemic and intrathecal chemotherapy

We have made good progress in recruiting patients with acute lymphoid leukemia (ALL) at the University of Kentucky Children's Hospital. To date, 20 children have been recruited. Prior to their beginning chemotherapy, we collected peripheral blood, as well as cerebrospinal fluid (CSF), and we also collected blood samples at the end of the induction phase and at days 1, 8, and 15 during the consolidation phase. Samples from all collections will be processed to determine changes in EVs that have occurred since the onset of cancer therapy. Among the 20 patients, samples from two patients were not appropriate for the study; thus, we plan to continue to recruit additional patients to complete the study.

We have begun experiments using samples from children. We use a very sensitive protein detection system, Jess, that enables us to detect several brain injury markers in EVs, even when they are present in small amounts, thus reducing the chance of false negative results. Our data indicate a trend for specific brain injury markers, GFAP, to increase following treatments, suggesting that glia cells were activated. These data will be validated once all the planned samples are collected.

Overall, the results thus far indicate potential activation of the brain- resident immune cells. We also have demonstrated the ability of an FDA-approved antioxidant, MESNA, to mitigate the effect of EVs to activate immune cells. Our final steps are to complete recruitment and to test whether the drug, MESNA, can prevent EVs isolated from our children cohort from activating immune cells. If the results confirm our initial findings, they will provide a science- based preclinical study for the development of future clinical trials.

Timeline:

Aims Check appropriate time period when each aim is completed	6 MO	12 MO	18 MO	24 MO	30 MO	36 MO	√
Initiate the studies proposed in Aim 1 using existing serum samples from children who received Doxorubicin treatment with and without MESNA							x
Obtain IRB approval to enroll pediatric patients							x
Stimulate innate immune cells of macrophage lineage and test the supernatants for production of anti- and pro-inflammatory cytokines		x	x	x	x	x	x
Characterize markers of brain injury in EVs isolated from children pre- and post-chemotherapy		x	x	x	x	x	
Recruit pediatric patients to collect sera and CSF for evaluation of the brain biomarker, 4-NHE-containing EVs, before and after chemotherapy, to identify products of oxidative tissue damage and to test the ability of EVs to induce pro-inflammatory cytokines		x	x	x	x	x	
Finalize the validation of EVs as biomarkers of brain injury in samples from pediatric patients and the effect of MESNA in prevention of EVs-mediated immune responses		x	x	x	x	x	
Write manuscript for publication							

Deliverables:

Check when deliverable is completed	√
Initiate the studies proposed in secondary objective #1 using existing serum samples from children who received Doxorubicin treatment with and without MESNA by December 31, 2018	x
Obtain IRB approval to enroll pediatric patients by December 31, 2018	x
Recruit pediatric patients to collect sera for evaluation of the 4-HNE-containing EVs, before and after chemotherapy, to identify products of oxidative tissue damage by December 31, 2019	x
Stimulate innate immune cells of macrophage lineage and test the supernatants for production of anti- and pro-inflammatory cytokines by December 31, 2019	x
Characterize markers of brain injury in EVs isolated from children pre- and post-chemotherapy by December 31, 2019	x
Finalize the validation of EVs as biomarkers of brain injury in samples from pediatric patients and the effect of MESNA in prevention of EVs-mediated immune responses by June 30, 2021	
Write manuscript for publication by June 30, 2021	

Quarterly Reports are due:

- October 15, 2020
- January 15, 2021
- April 15, 2021
- July 15, 2021

Reports should be returned to:

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