DISC2: Quest Review 21.1 – Review Summary

Application: DISC2-12400

Munjal M Acharya — University of California, Irvine
Stem cell-derived extracellular vesicles to reverse radiation-induced brain injury

Application #	DISC2-12400
Title (as written by the applicant)	Stem cell-derived extracellular vesicles to reverse radiation-induced brain injury
Research Objective (as written by the applicant)	These preclinical studies will discover the efficacy of stem cell-derived, nanoscale, extracellular vesicles (candidate) to treat adverse effects of cancer therapy on brain function and cognition.
Impact (as written by the applicant)	Stem cell-derived extracellular vesicles will address the confounders of stem cells (tumors, immunorejection, immunosuppression) & mitigate debilitating side-effects of cancer therapy on the brain.
Major Proposed Activities (as written by the applicant)	• Demonstrate the effectiveness of IV injections of stem cell-derived, nanoscale, extracellular-vesicles (EVs) to improve cognition in the mouse model of radiation- and chemo-therapy for brain cancers. • Determine the ability of EV treatment to protect against adverse effects of cancer therapy including neuro-inflammation, synaptic and micro-vascular damage in the brain. • Establish the neurocognitive benefits of injecting stem cell-derived-EV in brain cancer-bearing mice receiving combined radiation- and temozolomide chemo-therapy (CRT-TMZ). • Elucidate the impact of stem cell-derived-EV injections on neuropathological hallmarks of radiation- and chemo-therapy (TMZ) in the cancer-bearing mice brains. • Determine the safety and rule out the toxicity of stem cell-derived EV treatment in brain and peripheral organs in the mice receiving radiation- and chemo-therapy (TMZ) for brain cancer. • Confirm miRNA-124-based mechanism (commonly found within the EV cargo) of stem cell-derived EV-mediated neuroprotection in the mice undergoing radiation- and chemo-therapy for brain cancers.
Statement of Benefit to California (as written by the applicant)	In California, nearly 187,000 patients diagnosed with cancer will be alive in 5 years & more than 1.88 million have a history of cancer. Importantly, adult & childhood cancer survivors suffer from severe & persistent cognitive deficits that adversely affect their quality of life (QOL). A stem cell-based therapeutic could reduce inflammation & restore the cognitive function that may significantly improve patient's QOL, reduce financial hardship on patients, caregivers & the state of California.
Funds Requested	$1,064,724
GWG Recommendation	*(85-100): Exceptional merit and warrants funding, if funds are available*
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

Scoring Data

Final Score: 90

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Mean	91
Median	90
Standard Deviation	2
Highest	95
Lowest	88
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	0

Key Questions and Comments

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

GWG Votes	Does the proposal have the necessary significance and potential for impact?
Yes: 11	• The unmet medical need is clearly established in the introduction. The authors outline the prevalence of cognitive impairments as a result of cancer therapy. Currently, there are no available therapies to address this unintended consequence of cancer treatment. This need is particularly high after cancer treatment in children and patients with brain tumours, although many cancer patients will experience cognitive decline after treatment and would therefore benefit from this approach. • The applicant is pursuing two different approaches to the important problem of decreasing cognitive impairment after cranial radiotherapy and adjuvant chemotherapy. One of them, the use of extracellular vesicles, is scientifically interesting and provides the basis for an approach based on delivering a micro RNA (miR-124) that may be better suited for clinical development. • The possibility of preventing and reversing cognitive damage associated with radiation to the brain is an important one, and the demonstration that this is possible, particularly with a therapy that can be delivered through the vasculature, will greatly help to promote research in this field. • Childhood survivors of brain cancers can show reductions in I.Q. of up to 3 points. No clinical recourse is available yet to address this issue. Survivors of pediatric brain cancer and low-grade glioma (LGG) patients often have low quality of life.
No: 0	*none*

GWG Votes	Is the rationale sound?
Yes: 11	• The investigators have presented sound scientific rationale for the proposed project. They have described both the cognitive deficits observed in cancer survivors as well as provided information on the potential mechanisms driving these changes. They have additionally offered pre-clinical evidence that their proposed therapy can modify the underlying causes of cognitive decline in this patient group. • The rationale for this proposal has developed out of sound scientific experimentation that began with cell transplantation with neuroepithelial stem cells. The use of extracellular vesicles is a logical development from this beginning, and the progression to identification of a single micro RNA species that is part of the cargo of the extracellular vesicles is another promising step forward. • The EV-derived candidate miRNA-based mechanism they identified to ameliorate clinically relevant brain cancer therapy-induced cognitive impairments and neuroinflammation adds a layer of novel mechanism.
No: 0	*none*

GWG Votes	Is the proposal well planned and designed?

Yes: 11	• The provided preliminary data supports the feasibility of the proposed EV treatment in rats. It demonstrates that the team possesses the technical knowledge required to set up the glioma model, perform treatments and evaluate behavior and histopathology post-treatment.
	• The preliminary data are quite striking in demonstrating the ability of the extracellular vesicles to treat cognitive impairments and irradiated animals.
	• The ability to treat immunocompetent animals through a relatively noninvasive injection is a very surprising outcome that is also quite promising.
	• Strong preliminary data.
	• The applicant provides extensive preliminary data demonstrating their ability to produce hNSC-derived EVs. However, there is little information detailing how EV quality and homogeneity of EV cargo will be evaluated between batches. In addition, the investigator proposed to utilize EVs in the exosome size-range (40-100 nm) but no details are provided regarding how this particular EV population will be isolated after ultracentrifugation nor why it is specifically targeted.
	• A number of potential pitfalls identified by the applicant have been thoroughly addressed for each aim proposed. The applicants have clearly delineated the main limitations of their studies and suggested adequate alternatives. However, other important pitfalls and alternatives have not been identified and/or discussed:
	  ◦ It is well recognized that stem cell-based models frequently suffer from variability between cell passages. While the applicants describe a verification step for EV size, other factors such as expression levels of bioactive miRNA(s) and marker proteins would also be important to evaluate between batches. To this end, what are the criteria used to define a therapeutic EV? Is it based on miRNA composition?
	  ◦ In Aim 3, the applicant proposes an AAV approach to evaluate the neuroprotective potential of the miRNA. While this is an important aspect for validating the hNSC-derived EV therapeutic potential, the proposed experiment should include additional endpoint measures. For example, what will be the levels of the miRNA expression and how does it compare to EV-based cargo delivery? It seems unlikely that an overexpression model accurately recapitulates the miRNA levels from EV delivery. In addition, it is unclear which cell types uptake hNSC-derived EVs, thus proposing a broad AAV-based delivery may not mimic the EV mechanism of action.
	  ◦ The proposed experimental timeline indicates that EV injections will begin after CRT-TMZ treatment has already induced neuronal damage. Parallel experiments where EVs are injected as a pre-treatment to prevent neuronal damage could also be considered. This approach could be more beneficial for patients by directly preventing neuronal loss and neuroinflammation, instead of rescuing or attempting to regenerate cells after damage.
	  ◦ To understand the molecular effects of hNSC-derived EV treatment, the study could benefit from a single cell RNA-seq experiment to evaluate the response of individual cell types to EV injections (in healthy animals and after CRT-TMZ treatment).
	  ◦ It is unclear whether fibroblast-derived EVs are appropriate controls for this experiment. These EVs are likely to contain bioactive cargo which may trigger a detrimental response.
	• Overexpression of a miRNA could be problematic as miRNA have thousands of targets.

No: 0	*none*

GWG Votes	Is the proposal feasible?
Yes: 11	• The applicant described multiple different milestones relating both to behavioral phenotypes and to the assessment of inflammation and blood-brain barrier integrity. While the proposed work is substantial, the short duration of the experiments and the specificity of the post-mortem plan should facilitate completion within the stated timeline. • The options for progression are thoughtful in two ways. First, the ability to deliver extracellular vesicles via the vasculature and have beneficial effects is much more realistic than cell transplantation. Second, the ability to focus attention on a single micro RNA carried in the extracellular vesicles, which are difficult to develop commercially, provides an interesting option for further development. • The approach uses EV derived from already funded iPSC cells and aims are logical. Progression to EV-derived miRNA to reverse treatment induced cognitive dysfunction is a novel approach.
No: 0	*none*

GWG Votes	Does the project serve the needs of underserved communities?

Yes: 11	• The applicants have meticulously described the influence of race, ethnicity, sex and gender on cognitive impairments after cancer treatment. The incidence of cancer has been broken down into specific categories to provide an overview as to how this affects all sub-groups of individuals, in the world and in California as well. • This section of the proposal was extremely well justified. While most of these factors cannot directly be addressed in the current study design, sex has been considered and all experiments include both male and female mice. • There is no doubt that if the applicants are successful in bringing this to the clinic, all cancer patients affected by these major side-effects of chemo and radiation therapy would benefit from their treatment, especially younger patients who are likely to live with these debilitating cognitive affectations for many years. • The proposal mentioned that these detrimental side-effects are more prevalent in certain underserved racial/ethnic communities. While it is unclear where the supporting epidemiological data for this statement comes from, if a cost-effective therapy is produced it is likely to be of significant benefit to this population. However, the route of treatment delivery would have to be re-evaluated for humans but I believe this would be a minor obstacle to clinical translation. • Cognitive problems after cancer treatment do not segregate according to race, ethnicity or related variables. • Well addressed.
No: 0	*none*

Additional Review Information

Review and Scoring Process

Applications are scored on a scale that ranges from 1 – 100, with 100 being the highest achievable score.

Applications are separated into two funding tiers. An application's median score determines the funding tier as follows:

85-100 = exceptional merit and warrants funding, if funds are available
1-84 = not recommended for funding

The Review Report provides only a brief summary of the evaluation of your application by the GWG. The report is not an exhaustive critique and does not cover all of the factors that may have contributed to the final score or the final recommendation. The report provides information son how the GWG panel scored, how each review criterion influenced the score, and specific bulleted comments that reviewers provided following the discussion.

Response to Review

The GWG conducts the scientific evaluation of proposals submitted to CIRM. If the applicant (PI/PD) wishes to appeal the scientific review by the GWG, he/she may only do so based on a demonstrable conflict of interest. All appeal requests must be made through the CIRM Review Office within 10 days of CIRM making this report available.

Any questions related to the review should be addressed to Dr. Gil Sambrano (gsambrano@cirm.ca.gov) or Dr. Hayley Lam (hlam@cirm.ca.gov).

ICOC/Application Review Subcommittee Meeting

Funding decisions are made by the Application Review Subcommittee of CIRM's governing board, the Independent Citizens Oversight Committee (ICOC). The Subcommittee, which meets concurrently with the Board, is composed of 20 voting members (the Patient Advocates, Nurses, and Industry members of the Board, along with the Chair and statutory Vice Chair of the Board) and 15 non-voting members (the 15 members of the Board who are appointed from institutions that are eligible to receive CIRM funding).

The Applications Review Subcommittee will conduct a programmatic assessment of applications reviewed by the GWG. The Subcommittee may consider any factors (such as availability of funds, overall grant portfolio, RFA priorities, strategic considerations, the applicants' approach to issues of diversity, equity, and inclusion) that might impact on their decision to fund or not fund applications. The Subcommittee aims to fund applications that are both scientifically meritorious and that bring programmatic value to the CIRM portfolio.

Under California's open meeting laws, members of the public, including applicants for CIRM funding, may provide written and oral comments to the ICOC regarding items on the Board's agenda. Applicants may attend and observe the ICOC meeting. Applicants may contribute oral comments for not more than three (3) minutes during the public comment periods. The ICOC Chairman will announce the public comment period, which typically occurs prior to the Board's voting on any motion. Applicants may also provide written comments to the ICOC. All correspondence to the ICOC must be submitted to the Executive Director of the ICOC, Maria Bonneville, at mbonneville@cirm.ca.gov. Any correspondence to the ICOC that relates to an appeal of a funding recommendation by the GWG will be redirected to the CIRM Review Office (see "Response to Review" below).

Award Notification

If approved for funding, CIRM will notify you by email of the ICOC's funding decisions following the ICOC meeting.