KPCRTF State Funded Projects Reporting Template

University of Kentucky – Project #3

IND enabling studies of mithramycin derivatives for the treatment of Ewing sarcoma

Program Director: Markos Leggas

Reporting Period: April 2021– July 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.

Over the last period we have conducted in vivo toxicity and efficacy studies, but continued to observe variable toxicity, even with the new formulation which utilized non-toxic ingredients. We are currently exploring the possibility that during the chemical synthesis of different batches we are obtaining variable amounts of epimers (the same structure but one atom may have a different orientation in some molecules as compared to others in the same batch). We believe that one epimer may be contributing to the majority of the toxicity. This structural difference is very difficult to detect with conventional tests and we are attempting to crystalize material from different batches with x-ray crystallography. This will provide a definitive answer if both epimers crystalize but may not if only one epimer crystalizes. Separately, we have developed an additional chemical route for coupling the tryptophan molecule to the MTM-SA intermediate using newly available commercial reagents designed to minimize epimerization. With regard to the efficacy, the effect of MTMSA-Trp is being explored in three additional Ewing tumor cell lines but these studies are still ongoing.

Although the PCRTF grant funding period has been completed, we have secured funding from the NCI through an R01 grant that will support additional basic research questions around this project. Based on preliminary evidence, we also anticipate that the results of the ongoing efficacy study will be positive and plan to engage with the National Cancer Institute (NCI) Experimental Therapeutics (NExT) Program to further the development of MTMSA-Trp.

Aim 1: Obtain large quantities of MTMSA via industrial scale bacterial fermentation conducted by a contract research organization (CRO)

This aim has transitioned to generating MTMSA using our own chemical synthetic pathway using MTM which we purchased from a CRO.

We are currently generating crystals of MTMSA-Trp to perform x-ray crystallography and assess whether epimers are present. The production of crystals by typical means has been elusive and we are currently also attempting to generate crystals using metals that can complex two molecules together.

We have developed an additional synthetic approach to attach tryptophan (Trp) to MTMSA. This approach utilizes new chemical reagents designed to minimize the potential for epimerization.

Aim 2: Conduct defined pharmacology, toxicity and safety studies with MTMSA-A10 that are required for the submission of an investigational new drug (IND) application to the FDA

Our pharmacology and toxicology studies continue. We transitioned to a new formulation using a small percentage of surfactant and saline which have provided appropriate solubility and stability for the experiments we are conducting. We have determined that the maximum tolerated dose of the current batch is 3 mg/kg dosed daily for five days. This is lower than the original batch that was tolerated at 5.5 mg/kg but higher than a third batch that was only tolerated at 1.8 mg/kg. The 1.8 mg/kg did not yield any efficacy against tumors, but this was unexpected because the 5.5mg/kg batch was yielding results even when the dose was diluted to 1/5 (ie, 1.1mg/kg). In preliminary testing, the 3 mg/kg batch showed efficacy and we are currently proceeding with formally testing three additional Ewing sarcoma cell lines in mouse xenografts using this dose (3 mg/kg). The completion of these studies will provide evidence of the in vivo efficacy in a total of four Ewing cancer cell lines.

Timeline:

	Activity	6 MO	12 MO	18 MO	24 MO	√
Aim 1	Fermentation – large scale	CRO				Y
	Analytical (CRO)	CRO				Y
	Synthesis (CRO)	CRO				Y
	Small scale fermentation and synthesis	UK	UK	UK	UK	Y
	Activity	**6 MO**	**12 MO**	**18 MO**	**24 MO**	√
Aim 2	Pharmacology (efficacy)	UK	UK			ongoing
	Bioanalytical validation	UK				Y
	Pharmacokinetics/toxicokinetics/acute toxicity		UK	UK	UK	ongoing
	GLP Safety and Toxicity		CRO	CRO	CRO	

Deliverables:

Check when deliverable is completed	√
Perform synthesis of small batches of MTMSA-A10 for non-GMP animal pharmacology studies at UK by June 30, 2020	Y
Perform non-GMP animal pharmacology studies at UK by June 30, 2020	Y
Perform synthesis of the Tryptophan-Tryptophan dipeptide which will be used for synthesis of MTMSA-A10 by a CRO by September 2019	*Y
Work with a CRO for the scale-up, and purification of MTMSA by December 31, 2019	# Y
Engage a CRO for the scale-up, and purification of GMP grade MTMSA-A10 by March 31, 2020	#
Engage a CRO for the conduct of toxicity and safety studies by September 30, 2020	#

*We discovered that the activity of MTMSA-A10 (aka, MTMSA-Trp-Trp) is not as great in animals as it was in vitro. However, the backup molecule MTMSA-Trp has excellent pharmacological characteristics and we are proceeding the development of that molecule instead.
We are planning to engage with the NCI Experimental Therapeutics program to finalize the development of the compound, instead of the CRO. However, in verbal discussions, they have requested that we extensively explore the efficacy of the lead compound(s) in more than one cell line. These activities are ongoing.

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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