No.	K24-1117	
Project Title	Functional interplay between endogenous MLL1/KMT2A and MLL-fusion oncoproteins	
Principal Investigator	Patricia Ernst (University of Colorado/Anschutz Medical Campus・Professor)	
Project Member(s)	**IMSUT Host Researcher**	Atsushi Iwama (The Institute of Medical Science, The University of Tokyo・Professor)
	Project Member (s)	Patricia Ernst (University of Colorado/Asnchutz Medical Campus・Professor)
	Project Member (s)	Wenjuan Liao (University of Colorado/Anschutz Medical Campus・Postdoctoral Fellow)
	Project Member (s)	Akihiko Yokoyama (National Cancer Center Tsuruoka Metabolomics Laboratory・Professor)

(Form 2)

Principal Investigator
ERNST, Patricia

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Joint Research Report (Annual)

Annual Report

Report

Background, Significance and Premise: The MLL1 gene is characteristically rearranged (*MLL1*-r) to result in a fusion oncogene in >70% infant acute leukemia and 10% adult AML. This particular cytogenetic category in either group predicts poor response to chemotherapy 5-year survival rates below 50% despite advances in immunotherapy, bone marrow transplantation and targeted therapy. The lack of treatment options and the low mutational burden have motivated detailed mechanistic studies on fusion oncoprotein function. Such studies have yielded small molecule menin inhibitors, the first of which has achieved FDA approval in 2024. These new targeted therapeutic strategies are promising, but their optimal combination with other therapeutic strategies such immunotherapies is unclear and may vary depending on leukemia subtype. Further, the molecular interplay between *MLL*-r alleles and the endogenous WT *MLL1* may impact menin inhibitor effectiveness given the inhibitor also impacts the WT *MLL1* allele.

Progress this year: Due to the biochemical challenges of working with the MLL-AF9-driven AML as originally proposed, we switched our model system to 293T cells. We have generated a 293T Flip-in subclone containing a single copy of MLL-AF9 and then followed by Cas9-CRISPR mediated knock out of the endogenous MLL1 gene, both copies. Several subclones were confirmed, expanded and shipped to Dr. Iwama and Dr. Yokoyama. The goal with this cellular system is that we will monitor genomic occupancy of the MLL-AF9 fusion and gradually titrate back in the wild type MLL1 protein by transient transfection. This system will allow us to determine, genome-wide, whether endogenous MLL1 can compete with the fusion oncoprotein MLL-AF9. Dr. Yokoyama has already performed several studies to demonstrate that the HA tagged MLL-AF9 molecule is detectable on chromatin and we have demonstrated genome-wide transcriptional differences of the subclone with MLL1 knocked out as compared to the parental cell line.

Roles of collaborators: We have collaborated for many years with Dr. Yokoyama as he has is the best expertise of anyone in the world on the structure-function of MLL fusion oncoproteins. He has given us critical advice on molecular and biochemical approaches and has sent us multiple new plasmids encoding MLL-AF4 and other fusions. With Dr. Iwama, we have started a collaboration on a different B-ALL project, utilizing a surface bound version of IL-7 that enables B-lymphopoiesis. Dr. Iwama may be involved in the genome-wide sequencing of chromatin immunoprecipitation data also

Specific meeting with collaborators and plans for travel: We currently meet with Drs. Yokoyama and Iwama frequently by zoom to discuss research applications and work on incorporating the developing work into manuscripts. We may plan travel in the near future to present this collaborative work.