AWARD NUMBER: W81XWH-13-1-0340

TITLE: Zebrafish Functional Genetics Approach to the Pathogenesis of Well-Differentiated Liposarcoma

PRINCIPAL INVESTIGATOR: Alejandro Gutierrez, MD

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Fort Detrick, Maryland 21702-5012

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**Zebrafish Functional Genetics Approach to the Pathogenesis of Well-Differentiated Liposarcoma**

During this award, we performed all work proposed under Aim 1 of our original proposal to test the hypothesis that FRS2 is a 12q oncogene that activates oncogenic signal transduction, using FRS2 overexpression in genetically engineered zebrafish models and in normal human preadipocytes. Aim 2 was also completed, and demonstrates that MDM2, CDK4 and HMGA2 are bona fide liposarcoma oncogenes and licensed therapeutic targets. This work provides a compelling rationale for testing small molecule inhibitors of these oncogenes in patients with well-differentiated liposarcoma, for whom there are currently no effective medical therapies.

15. **SUBJECT TERMS:** Well-differentiated liposarcoma, oncogene, zebrafish, genetically engineered animal models, cancer therapy

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   - c. THIS PAGE: U

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19a. **NAME OF RESPONSIBLE PERSON:** USAMRMC

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1. **INTRODUCTION:**

Well-differentiated liposarcoma is the most common soft-tissue sarcoma of humans, and predisposing factors include exposure to dioxin-containing herbicidal agents used during the Vietnam War (Agent Orange) and radiation from Atomic Bombs (Institute of Medicine 1994; Kogevinas 1997; Preston 2007). The molecular pathogenesis of this disease is very poorly understood, and there are no effective medical therapies for patients whose tumors cannot be fully removed at surgery (Dalal 2008). Current knowledge of well-differentiated liposarcoma pathobiology has been driven by descriptive analyses of human tumors, which have revealed that almost all cases harbor amplifications of chromosomal material from 12q (Suijkerbuijk 1994; Pedeutour 1999). These amplicons consistently involve the MDM2, CDK4 and HMGA2 genes, which have long been hypothesized to play a pathogenic role in this disease. Several additional genes are also involved within these amplicons, some of which have also been proposed to be pathogenic. However, the lack of animal models of this disease has made it impossible to identify the key oncogenes that drive selection for these amplifications, and therefore are expected to represent the “Achilles’ Heels” of this disease. Indeed, the products of such genes could offer optimal targets for therapeutic intervention, in contrast to non-pathogenic “passenger” genes which are amplified only because of their physical proximity to bona fide oncogenes. The difficulty of distinguishing driver from passenger genes has resulted in a dearth of functionally validated therapeutic targets, representing a major obstacle to the development of effective cancer therapies. We recently developed the first animal model of well-differentiated liposarcoma, induced by expression of a constitutively active AKT transgene in mesenchymal progenitors of p53-mutant zebrafish (Gutierrez 2011). Thus, this animal model now allows the performance of pioneering in-depth investigation of the molecular pathogenesis underlying well-differentiated liposarcoma, which was the focus of this project.

2. **KEYWORDS:**

Well-differentiated liposarcoma, Oncogene, Zebrafish, Genetically engineered animal models, Cancer Therapy

3. **ACCOMPLISHMENTS:** Major Goals and Accomplishments during the award period are summarized here with respect to each task outlined in the approved Statement of Work, as follows:

3A. **Specific Aim 1.** Test the hypothesis that FRS2 overexpression drives aberrant PI3K-AKT activation in well-differentiated liposarcoma.
3A.1. Task 1. Test whether FRS2 is a well-differentiated liposarcoma oncogene in transgenic zebrafish (timeframe, months 1-12).

**Subtask 1a. Test whether FRS2 overexpression in zebrafish mesenchymal progenitors is sufficient to induce well-differentiated liposarcoma (timeframe, months 1-6).** We generated a cohort of zebrafish expressing a rag2:FRS2 transgene that drives FRS2 overexpression in mesenchymal progenitors. Positive controls were injected with rag2:myr-Akt2, and negative controls were injected with rag2:EGFP. In our original application, we proposed generating and testing a minimum of 50 zebrafish per condition, based on calculations of our biostatistician collaborator Dr. Kristen Stevenson. We analyzed a cohort of 81 rag2:FRS2 zebrafish, as well as 58 positive controls and 61 negative controls, for a minimum follow-up period of 6 months (range, 6-11 months), but we saw no liposarcomas develop in FRS2-transgenic animals (Figure 1A). Subtask 1a was completed as planned.

**Subtask 1b. Test whether FRS2 overexpression in zebrafish mesenchymal progenitors collaborates with p53 mutation in well-differentiated liposarcoma pathogenesis (timeframe, months 3-9).** We also generated a cohort of rag2:FRS2-transgenic zebrafish in the p53 homozygous mutant background, to test whether FRS2 overexpression collaborates with p53 mutations. We analyzed a cohort of 91 rag2:FRS2, p53-mutant zebrafish for tumor onset for a minimum of 6 months (range, 6-8 months), as well as a cohort of rag2:myr-Akt2 positive controls and rag2:EGFP negative controls (>50 fish in each group), but also saw no liposarcomas develop in FRS2-transgenic animals (Figure 1B). Subtask 1b was completed as planned.

**Figure 1. FRS2 is not sufficient to induce well-differentiated liposarcoma.** To test whether FRS2 is a liposarcoma oncogene, we generated transgenic zebrafish in the p53 wild-type (A) or p53 homozygous-mutant background (B), which expressed rag2:FRS2, rag2:myr-AKT2 (positive control), or rag2:GFP (negative control) transgenes. This experiment revealed no tumors in FRS2-transgenic zebrafish indicating that FRS2 expression alone is not sufficient to drive liposarcoma development. By contrast, tumors in myr-AKT2 positive controls occurred at the predicted rate based on our previous findings (Gutierrez 2011).
Subtask 1c. Test whether FRS2 overexpression in zebrafish mesenchymal progenitors drives aberrant PI3K-AKT and RAS-MAPK pathway activation (timeframe, months 9-12). We analyzed a cohort 4 of zebrafish expressing rag2:FRS2, as well as rag2:GFP negative control, and rag2:KRAS-G12D (encoding constitutively active Kras) positive control, for evidence of PI3K-AKT pathway activation (as assessed using immunohistochemistry for phospho-AKT (Ser473), and of RAS-MAPK pathway activation (using immunochemistry for phospho-ERK1/2 (Thr202/Tyr204). Immunohistochemical analysis revealed no evidence of PI3K-AKT or RAS-MAPK pathway activation by FRS2, whereas positive control zebrafish expressing an activated KRAS allele showed activation of these pathways (Figure 2). Subtask 1c was completed as planned.

![Figure 2. FRS2 does not activate PI3K-AKT or RAS-MAPK signaling in zebrafish adipocytes or mesenchymal progenitors. Hematoxylin and Eosin (H&E) staining or immunohistochemistry for phospho-AKT(S473) or phospho-ERK(T202/Y204) was performed in zebrafish expressing rag2:GFP (negative control), rag2:FRS2, or rag2:KRAS(G12D), encoding a constitutive RAS allele.](image)

3A.2. Task 2. Test whether FRS2 overexpression in normal human preadipocytes promotes proliferation and oncogenic signal transduction (timeframe, months 1-12).

For all experiments in this Task, normal human preadipocytes were obtained from a commercial provider (www.promocell.com, product # C-12730). The human liposarcoma cell lines we used are LPS 141, LPS 510, LPS 789, LPS 853, and T449, which were obtained from the laboratory of my co-mentor Dr. Jonathan Fletcher, where most of these lines were originally derived. Both
normal preadipocytes and human liposarcoma cell lines were provided to us anonymously without any linking identifiers, and it is impossible for us to determine the identity of the original source.

Subtask 2a. Test whether FRS2 overexpression in normal human preadipocytes promotes proliferation and overcomes senescence (timeframe, months 1-6). We generated a lentivirus construct expressing full-length human FRS2, and used it to successfully infect normal human preadipocytes with this gene. EGFP was the negative control, and KRAS-G12D was the positive control. We then analyzed these cells for growth, proliferation, and apoptosis, but found no effect of FRS2 overexpression in these cells (Figure 3). Subtask 2a was completed as planned.

Subtask 2b. Test whether FRS2 overexpression in normal human preadipocytes drives oncogenic signal transduction (timeframe, months 3-9). We also analyzed the cells generated in Subtask 2a for biochemical evidence of activation of PI3K-AKT and RAS-MAPK pathways, using Western blot analysis for phosphorylation of AKT, S6K, MEK and ERK. This experiment revealed no evidence of activation of these signal transduction pathways (Figure 4) and this Subtask was completed as planned.
Subtask 2c. Test whether FRS2 is required for proliferation and survival of human liposarcoma cells (months 12-24). We performed shRNA knock-down in human cell lines derived from patients with well-differentiated liposarcoma that harbor amplification and overexpression of FRS2, and found that FRS2 knock-down specifically decreases cell viability and induces cell death through the mitochondrial pathway of apoptosis (Figure 5). Subtask 2c was completed as planned.

3A.3. Task 3. Test the hypothesis that growth, survival, and oncogenic signal transduction in cell lines derived from patients with well-differentiated liposarcoma is dependent on FRS2 overexpression (timeframe, months 1-12)

Subtask 3a. Identify shRNA hairpins that effectively silence FRS2 expression in human liposarcoma cells in a conditional fashion (timeframe, months 1-3). We have identified two distinct shRNA hairpins that effectively silence FRS2 expression in human liposarcoma (Figure 5A), and successfully cloned these into a doxycycline-inducible vector. Subtask 3a was completed as planned.

Subtask 3b. Test whether FRS2 knock-down in human liposarcoma cell lines impairs proliferation and oncogenic signal transduction (timeframe, months 3-9). We performed shRNA knock-down of FRS2, and found that this impairs viability and signaling through key downstream oncogenic signaling pathways, including the PI3K-AKT and MAPK pathways, as assessed by Western blot for phosphorylation of key downstream kinases including AKT, MEK and ERK (Figure 5B-C). Subtask 3b was completed as planned.
Subtask 3c. Investigate the hypothesis that FRS2-dependent signal transduction requires an activated kinase (timeframe, months 9-24). We undertook a candidate approach and performed shRNA knock-down of three kinases known to interact with FRS2: FGFR, RET and TRKA. shRNA knock-down was performed in human liposarcoma cell lines, in order to test the hypothesis that one of these kinases is required for FRS2-dependent signal transduction. However, shRNA knock-down of these three kinases had no significant effect on AKT and ERK pathway activation in these cells (data not shown), suggesting that these are not the relevant kinases in this model. Two other kinases have been reported to interact with FRS2, ALTK and TRKB, but neither of these genes are expressed in liposarcoma cell lines. These experiments thus indicate that FRS2-dependent signal transduction is mediated by a novel FRS2 interacting partner. To identify such an unknown partner, we are pursuing a mass spectrometry approach based on FRS2 pull-down analysis and mass spectrometry proteomics. Although we completed the initial phase of this Subtask involving knockdown of known FRS2-interacting proteins, we have not yet succeeded in identifying the relevant FRS2 binding partner, and these efforts will continue in our laboratory beyond the funding period of this award. Of note, we anticipated that finding the relevant kinase might very well extend beyond the funding period, and this was presented as such in our original proposal.
3B. Specific Aim 2. Test the hypothesis that MDM2, CDK4 and HMGA2 are well-differentiated liposarcoma oncogenes that collaborate synthetically in its molecular pathogenesis (timeframe, months 12-24).

3B.1. Task 1. Test the ability of MDM2 to accelerate the onset of AKT-induced well-differentiated liposarcoma (timeframe, months 12-18).

MDM2 is a ubiquitin ligase best-known as an inhibitor of p53 activity, and is consistently amplified in human well-differentiated liposarcoma. To test whether MDM2 can accelerate the onset of AKT-induced liposarcoma, we generated transgenic zebrafish expressing myristolated (constitutively active) AKT and co-expressing either MDM2 or GFP control. Expression of MDM2 significantly accelerated tumor onset in this model, proving that MDM2 is an oncogene in this disease (Figure 6). Although this was an expected finding, this was not previously proven, and this work validates MDM2 as a therapeutic target in this disease. The clinical relevance of this work is that small-molecule MDM2 inhibitors are now entering clinical trials, and could prove particularly useful for these patients.

The finding that human liposarcomas consistently inactivate the p53 pathway by MDM2 amplification, and not by mutating p53 which is much more common in other human cancers, suggests that MDM2 may also have oncogenic roles in this disease. To test this possibility, we are currently testing whether MDM2 can accelerate tumor onset in p53-null zebrafish. Although this experiment is not yet mature enough to allow firm conclusions to be drawn, preliminary data suggest that MDM2 retains significant activity as an oncogene even in p53-null zebrafish, consistent with the very interesting possibility that MDM2 has p53-independent oncogenic roles in this disease. This represents an entirely unexplored area of cancer biology that represents one major focus of our future work in this area.

Figure 6. MDM2 accelerates the onset of AKT-induced liposarcoma.
The incidence of liposarcoma was assessed in zebrafish expressing a constitutively active myr-AKT transgene, and either MDM2 or GFP (controls). MDM2 expression significantly potentiates AKT-induced liposarcoma, thus providing proof that MDM2 is an oncogene.
3B.2. Task 2. Test the hypothesis that CDK4 and HMGA2 are well-differentiated liposarcoma oncogenes (timeframe, months 18-24).

3B.3. Task 3. Test the hypothesis that YEATS4 is a non-pathogenic passenger gene within the liposarcoma 12q amplicons (timeframe, months 12-24).

Results for Tasks 2 and 3 are shown together in Figure 7. Generation of transgenic zebrafish expressing either CDK4 or HMGA2 in mesenchymal progenitors lead to tumor formation in both cases, implicating both of these genes as bona fide liposarcoma oncogenes. By contrast, no tumors were seen in any of the 50 zebrafish expressing the rag2-YEATS4 transgene, suggesting that this gene is indeed a non-pathogenic passenger. These tasks were completed as planned.

Opportunities for Training and Professional Development: This award has allowed me to formalize a mentoring relationship with my co-mentors on this project Drs. Thomas Look (Dana-Farber Cancer Institute) and Jonathan Fletcher (Brigham & Women’s Hospital). Although the formal award period has ended, I have built a lasting mentoring relationship with both Drs. Look and Fletcher, which will help guide my continued scientific development and success for years beyond the formal award period. I am most grateful for the DOD for this opportunity.

How Were the Results Disseminated to Communities of Interest: A portion of these results has been presented at national and international meetings, including Oral Platform Presentations at the 2013 International Conference on Sarcoma Biology (New York, NY), and an Oral Platform Presentation at the 2015 Annual Meeting of the American Association for Cancer Research. Once our studies are complete, they will be submitted for publication in peer-reviewed scientific journals.
What do you plan to do during the next reporting period to accomplish these goals: Nothing to report.

4. IMPACT:

Impact on the Development of the Principal Discipline of the Project:

This project aimed to leverage the first animal model of well-differentiated liposarcoma, the most common sarcoma of humans, to investigate the molecular pathogenesis of this disease and yield insights into novel therapeutic strategies. Previous work had revealed a series of candidate oncogenes that are recurrently amplified in the human tumors, but the lack of animal models severely impairs our ability to investigate the relevance of these candidates to the biology of the disease. The impact of this work is that it now provides, for the first time, functional evidence in a relevant in vivo model system that now allows us to identify bona fide liposarcoma oncogenes, which are optimal therapeutic targets, and distinguish these from non-pathogenic passengers that are amplified only due to their physical proximity to key oncogenes.

The impact of the work supported by this DOD award can be summarized based on the following conclusions:

- Although FRS2 is required for cellular viability and for activation of the PI3K-AKT and RAS-MAPK pathways in liposarcoma, the ectopic overexpression of FRS2 is not sufficient to activate oncogenic signaling in these cells. Although our hypothesis was proven incorrect, these results are nevertheless important because therapeutic strategies are typically most efficacious when they target a key oncogene whose aberrant activation directly drives oncogenic signaling. Our data suggest that although there may be some therapeutic benefit to targeting FRS2-dependent signaling in this disease, they do temper enthusiasm and suggest the need to prioritize the discovery of alternative candidate targets that are bona fide oncogenes.
- MDM2 and CDK4 are bona fide oncogenes and licensed therapeutic targets in well-differentiated liposarcoma. This is a highly clinically relevant finding because a number of MDM2 and CDK4 small molecule inhibitors are currently in late stages of clinical development, thus our results indicate that patients with well-differentiated liposarcoma could benefit significantly from these therapies, and should be prioritized in clinical trials of these drugs. Future efforts will be focused on testing the therapeutic activity of these drugs, alone and in combination, in our zebrafish model and other relevant preclinical models.
- Our results suggest that YEATS4 is a non-pathogenic passenger in liposarcoma, which is recurrently amplified in human tumors only because of its physical proximity to other oncogenes on chromosome 12q.
- HMGA2 is a particularly potent liposarcoma oncogene, whose ability to induce tumors in p53 wild-type zebrafish is similar to the combination of MDM2 and AKT combined. This suggests that HMGA2 amplification and overexpression play a key role in the pathogenesis of this disease. HMGA2 is a non-histone chromosomal protein whose functions are not well-understood, but include regulation of the enhanceosome and transcriptional output. How this gene drives sarcomagenesis is entirely unknown at the moment, and unraveling the role
of HMGA2 in liposarcoma pathobiology represents a very exciting and productive avenue for further investigation.

**Impact on Other Disciplines:** Although the primary impact of the work is on the biology and therapy of well-differentiated liposarcoma, the finding that HMGA2 is a particular powerful oncogene has implications for the basic biology of the enhanceosome and transcriptional regulatory mechanisms, as it indicates that amplification of a core enhanceosome component can be oncogenic. We anticipate that further investigation will provide major opportunities to unravel the role of the enhanceosome in human oncogenesis.

**Impact on Technology Transfer:** Nothing to report.

**Impact on Society Beyond Science and Technology:** This project has major implications for clinical medicine, as it provides compelling data to support clinical trials testing MDM2 and CDK4 inhibitors (which are currently available) in patients with well-differentiated liposarcoma, for whom there are no effective alternative therapies. The military impact is that liposarcoma is particularly common in military veterans exposed to Agent Orange, and is also a potential consequence of radiation exposure from military or terrorist attacks, thus development of effective therapies for this disease has high potential impact for both military personnel and civilians at risk of such exposures.

5. **CHANGES/PROBLEMS:** Nothing to report.

6. **PRODUCTS:**

**Publications, conference papers, and presentations:** A portion of this work was presented at the 2013 International Conference on Sarcoma Biology (New York, NY), and at the 2015 Annual Meeting of the American Association for Cancer Research (Philadelphia, PA). These presentation are noted in my curriculum vitae, included as Appendix I to this annual report.

7. **PARTICIPANTS AND OTHER COLLABORATING INSTITUTIONS:**

**Participants:**

Name: Alejandro Gutierrez  
Project Role: Principal Investigator  
Researcher Identifier: ORCID 0000-0002-0249-9007  
Nearest person month worked over the 2 years of the award: 4.8 months  
Contribution to project: Dr. Gutierrez has been responsible for directing all aspects of this project. This includes designing experiments, performing technically challenging aspects of key experiments, and interpreting data and results.

**Funding Support:** Research grants from the National Institutes of Health/National Cancer Institute, USC Parker Institute, Gabrielle’s Angel Foundation for Cancer Research, Damon Runyon Cancer Research Foundation, Linde Family Foundation, Boston Children’s Hospital.

Name: Christine Reynolds
Project Role: Research Technician  
Researcher Identifier: N/A  
Nearest person month worked over the 2 years of the award: 0.6 months  
Contribution to project: Ms. Reynolds has been responsible for carrying out key technical aspects of this proposal, particularly with respect to cloning and testing of the constructs for Aim 2. Ms. Reynolds is also our lab manager and managed ordering and supplies for this project.  
Funding Support: Research grants from the National Institutes of Health/National Cancer Institute, USC Parker Institute, Gabrielle’s Angel Foundation for Cancer Research, Damon Runyon Cancer Research Foundation, Linde Family Foundation, Boston Children’s Hospital.

Name: Alice Zi Wei Liao  
Project Role: Research Technician  
Researcher Identifier: N/A  
Nearest person month worked over the 2 years of the award: 0.6 months  
Contribution to project: Ms. Liao took over all of Ms. Reynolds’ responsibilities after Ms. Reynolds left the laboratory in mid-2015. Ms. Liao also functioned as our lab manager and managed ordering and supplies for this project.  
Funding Support: Research grants from the National Institutes of Health/National Cancer Institute and Boston Children’s Hospital.

Name: Oscar Calzada  
Project Role: Research Technician  
Researcher Identifier: N/A  
Nearest person month worked: 21 months  
Contribution to project: Mr. Calzada has been primarily responsible for the performance of all of the work in this proposal, with the assistance of Ms. Reynolds as detailed above, from the start of the project through mid-2015. Mr. Calzada was also involved in designing the experiments and interpreting the results.  
Funding Support: N/A

Name: Salmaan Karim  
Project Role: Research Technician  
Researcher Identifier: N/A  
Nearest person month worked: 3 months  
Contribution to project: Mr. Karim took over all of Mr. Calzada’s duties after Mr. Calzada left the laboratory in mid-2015. Mr. Karim was also involved in designing the experiments and interpreting the results.  
Funding Support: N/A

Changes in the Active Other Support of the PI:

1. Grants that have expired since start of the current award:

  TITLE: Zebrafish Chemical and Classical Genetics Approach to the Pathogenesis of T-ALL  
TIME COMMITMENT: 75%  
AGENCY: NIH 5K08 CA133103-04
PERFORMANCE PERIOD: 07/01/08 – 06/30/13

TITLE: Discovery and Targeting of Apoptosis Resistance Mechanisms in T-ALL
TIME COMMITMENT: 10%
AGENCY: NIH 1R21 CA167124-01
PERFORMANCE PERIOD: 06/01/12 – 05/31/14

TITLE: Unraveling the Molecular Pathogenesis of T-Cell Acute Lymphoblastic Leukemia using Zebrafish Genetics and Small Molecule Screens
TIME COMMITMENT: 10%
AGENCY: American Society of Hematology
PERFORMANCE PERIOD: 07/01/08 – 06/30/13
LEVEL OF FUNDING: $104,140 (annual direct costs)

TITLE: Molecular mechanisms of chemotherapy resistance in T-cell acute lymphoblastic leukemia
TIME COMMITMENT: 5%
AGENCY: Boston Children’s Hospital
PERFORMANCE PERIOD: 07/01/13 – 06/30/15
OVERLAP: NONE.

TITLE: The role of JAK3 mutations in T-ALL
TIME COMMITMENT: 10%
AGENCY: Linde Family Foundation
PERFORMANCE PERIOD: 06/01/14 – 05/31/15
OVERLAP: NONE.

2. New grants received since start of the current award:

TITLE: Therapeutic Activation of the PP2A Tumor Suppressor for High-Risk T-ALL
TIME COMMITMENT: 20%
AGENCY: USC Parker Institute
PERFORMANCE PERIOD: 01/01/13 – 12/31/15
OVERLAP: NONE.

TITLE: Therapeutic Activation of the PP2A Tumor Suppressor in High-Risk T-cell Acute Lymphoblastic Leukemia
TIME COMMITMENT: 2.5%
AGENCY: Gabrielle’s Angel Foundation
PERFORMANCE PERIOD: 05/30/13 – 05/29/16
OVERLAP: NONE.

TITLE: Mechanisms and Therapeutic Targeting of EZH2-Dependent Chemoresistance in T-ALL
TIME COMMITMENT: 20%
AGENCY: Damon Runyon Cancer Research Foundation
PERFORMANCE PERIOD: 07/01/13 – 06/30/17
OVERLAP: NONE.

TITLE: Pathobiology and Therapeutic Targeting of EZH2-Dependent Chemoresistance in T-ALL
TIME COMMITMENT: 20%
AGENCY: National Institutes of Health/National Cancer Institute
PERFORMANCE PERIOD: 09/01/15 – 08/31/20
OVERLAP: NONE.

TITLE: Translational Investigator Service Award
TIME COMMITMENT: 1%
AGENCY: Boston Children’s Hospital
PERFORMANCE PERIOD: 06/01/15 – 05/31/20
OVERLAP: NONE.

Other Organizations Involved as Partners: Not applicable.

8. SPECIAL REPORTING REQUIREMENTS: Not applicable.

9. APPENDICES:

Appendix I contains references cited.

Appendix II contains an updated curriculum vitae of the principal investigator, and highlighted in yellow is the presentation of a portion of the work funded by this award at the 2013 International Conference on Sarcoma Biology (New York, NY), and the 2015 Annual Meeting of the American Association for Cancer Research (Philadelphia, PA).
APPENDIX I - REFERENCES

Dalal KM, Antonescu CR and Singer S "Diagnosis and management of lipomatous tumors." J Surg Oncol (2008);97:298-313. PMCID:


Suijkerbuijk RF, Olde Weghuis DE, Van den Berg M, Pedeutour F, Forus A, Myklebost O, Glier C, Turc-Carel C and Geurts van Kessel A "Comparative genomic hybridization as a tool to define two distinct chromosome 12-derived amplification units in well-differentiated liposarcomas." Genes Chromosomes Cancer (1994);9:292-5. PMCID:
APPENDIX II – Updated Curriculum Vitae of the Principal Investigator

Date Prepared: December 11, 2015
Name: Alejandro Gutierrez
Office Address: Boston Children’s Hospital, Karp 8
300 Longwood Ave
Boston, MA 02115
Home Address: 81 Franklin St
Brookline, MA 02445
Work Phone: 617-919-3660
Work Email: alejandro.gutierrez@childrens.harvard.edu
Fax: 617-730-0934
Place of Birth: Montreal, Canada

Education
1996  B.A.  Chemistry  University of Arizona
2001  M.D.  Medicine  University of Pennsylvania School of Medicine

Postdoctoral Training
2001-2004  Resident  Pediatrics  The Children’s Hospital of Philadelphia
2004-2007  Clinical Fellow  Pediatric Hematology/Oncology  Dana-Farber Cancer Institute, Boston Children’s Hospital and Harvard Medical School
2007-2012  Postdoctoral Research Fellow  A. Thomas Look Laboratory  Dana-Farber Cancer Institute

Faculty Academic Appointments
2007-2012  Instructor  Pediatrics  Harvard Medical School
2012-  Assistant Professor  Pediatrics  Harvard Medical School

Appointments at Hospitals/Affiliated Institutions
2007-  Staff physician  Hematology/Oncology  Boston Children’s Hospital
2007-  Staff physician  Pediatric Oncology  Dana-Farber Cancer Institute
2012-  Principal Investigator  Hematology/Oncology  Boston Children’s Hospital
2012-  Member  Leukemia and Sarcoma Programs  Dana-Farber/Harvard Cancer Center
2015- Member Immunology Graduate Program Harvard Medical School
2015- Associate Member Broad Institute of MIT and Harvard

**Other Professional Positions**

<table>
<thead>
<tr>
<th>Year</th>
<th>Position Description</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012-2014</td>
<td>Member, Project Advisory Committee</td>
<td>World Child Cancer Foundation</td>
</tr>
<tr>
<td>2013-</td>
<td>Affiliated Faculty of Advisors, Academy of Investigation</td>
<td>Boston Combined Residency Program in Pediatrics</td>
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</tbody>
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**Major Administrative Leadership Positions**

- **Local**
  - 2012-2016 Co-Director, Pediatric Hematology/Oncology Research Seminar Series Boston Children’s Hospital/Dana-Farber Cancer Institute

**Committee Service**

- **Local**
  - 2012-2013 Member, Faculty Search Committee Dana-Farber Cancer Institute, Boston Children’s Hospital, Harvard Medical School
  - 2013-2014 Member, Faculty Search Committee Boston Children’s Hospital, Dana-Farber Cancer Institute, Harvard Medical School
  - 2014-2015 Member, Faculty Search Committee Dana-Farber Cancer Institute, Boston Children’s Hospital, Harvard Medical School
  - 2015- Member, Fellowship Selection Committee Pediatric Hematology/Oncology Fellowship Program, Dana-Farber/Boston Children’s Cancer and Blood Disorders Center

**Professional Societies**

- 2004- American Society of Pediatric Hematology/Oncology Member
- 2007- American Society of Hematology Member
  - 2012 Annual Meeting: Abstract Reviewer and Scientific Chair, Oral Abstract Session
  - 2013 Annual Meeting: Abstract Coordinating Reviewer and Scientific Chair, Oral Session
  - 2014 Annual Meeting: Scientific Chair, Oral Abstract Session
- 2011- American Society of Clinical Oncology Member
  - 2011 Annual Meeting: Scientific Chair, Oral Abstract Session
- 2011- Children’s Oncology Group Member
- 2011- American Association for Cancer Research Member
- 2013- Society for Pediatric Research Elected member

**Grant Review Activities**

- 2012 FWO (Belgian National Fund for Scientific Research) Ad Hoc Reviewer
- 2013 Flemish League Against Cancer Ad Hoc Reviewer
- 2014 Association for International Cancer Research Ad Hoc Reviewer
- 2015 Children with Cancer UK Ad Hoc Reviewer
- 2015 German-Israeli Foundation for Scientific Research Ad Hoc Reviewer
Editorial Activities

Ad Hoc Reviewer
Blood
Cancer Research
FEBS Journal
Haematologica
Journal of Clinical Oncology
Laboratory Investigation
Leukemia
Molecular Cancer Therapeutics
Nature Medicine
Pediatric Blood & Cancer
Scientific Reports

Other Editorial Roles
2015 Guest Associate Editor PLOS Genetics

Honors and Prizes
2009 ASH-AMFDP Scholar Award American Society of Hematology
2010 Young Investigator Award American Society of Pediatric Hematology/Oncology
2013 Research Fellow Award Gabrielle’s Angel Foundation for Cancer Research
2013 Elected Member Society for Pediatric Research
2014 Clinical Investigator Award Damon Runyon Cancer Research Foundation
2014 Investigatorship Boston Children’s Hospital

Report of Funded and Unfunded Projects

Funding Information

Past
2005-2008 Pathophysiology of Human Blood Cells (PI: Lux)
NIH/NHLBI T32 HL007574
This training grant supported my early postdoctoral training in cancer genetics.

2007-2010 A Zebrafish-Based Screen for New Drugs for the Treatment of T-Cell ALL
William Lawrence Foundation Research Grant
PI ($400,560)
The goals of this project are to perform a synthetic lethal screen in transgenic zebrafish for small molecules that are synthetically lethal to MYC overexpression in developing lymphocytes, and to pursue the preclinical development of these lead compounds into novel anticancer agents for the treatment of T-ALL and other MYC-related cancers.
2008-2013 Zebrafish Chemical and Classical Genetics Approach to the Pathogenesis of T-ALL  
NIH/NCI K08 CA133103  
PI ($704,700)  
The major goals of the project supported by the career development award are to exploit the zebrafish model system to identify pathways that are required for the survival of MYC-overexpressing cells, and to establish the functional role of genes involved in recurrent T-ALL genomic alterations as potential novel T-ALL oncogenes and tumor suppressors.

2009-2013 Unraveling the Molecular Pathogenesis of T-Cell Acute Lymphoblastic Leukemia using Zebrafish Genetics and Small Molecule Screens  
ASH-AMFDP Career Development Award  
PI ($416,560)  
The major goals of this career development award are to perform a small molecule screen in transgenic zebrafish to identify targetable genes and pathways that are selectively required for the survival of MYC-overexpression thymocytes, and to establish the functional relevance of genes involved in recurrent T-ALL genomic alterations to the pathogenesis of T-ALL using zebrafish genetic models.

2010-2011 Molecular Targets in Well-Differentiated Liposarcoma  
Harvard Catalyst Pilot Grant  
PI ($50,000)  
The goal of this project is to exploit functional genetic analyses in a zebrafish model of well-differentiated liposarcoma to identify novel therapeutic targets in this chemoresistant disease.

2012-2014 NIH/NCI R21 CA167124  
PI ($275,000)  
The goal of this project is to establish molecular mechanisms mediating apoptosis resistance in high-risk subsets of T-cell acute lymphoblastic leukemia, and to develop molecularly targeted therapies to restore chemosensitivity.

Department of Defense CA120215  
PI ($240,000)  
The goal of this project is to exploit zebrafish functional genetics to define the oncogenes that drive selection for the characteristic 12q amplifications in human well-differentiated liposarcoma.

2013-2015 Molecular Mechanisms of Chemotherapy Resistance in T-ALL  
Boston Children’s Hospital Faculty Career Development Fellowship  
PI ($100,000)  
The goal of this project is to define the genetic lesions that underlie failure of conventional chemotherapy in T-cell acute lymphoblastic leukemia.

Current  
2013-2015 Therapeutic Activation of the PP2A Tumor Suppressor for High-Risk T-ALL  
William Lawrence & Blanche Hughes Foundation
This project aims to unravel the structural basis of PP2A activation by perphenazine, to define the mechanism through which PP2A activation kills T-ALL cells, and to test the hypothesis that pharmacologic PP2A activation will represent effective therapy for human treatment-resistant T-ALL.

2013-2016 Therapeutic Activation of the PP2A Tumor Suppressor for High-Risk T-ALL
Gabrielle’s Angel Foundation
PI ($225,000)
This project aims to unravel the structural basis of PP2A activation by perphenazine, to define the mechanism through which PP2A activation kills T-ALL cells, and to test the hypothesis that pharmacologic PP2A activation will represent effective therapy for human treatment-resistant T-ALL.

2014-2017 Pathobiology and Therapeutic Targeting of EZH2-Dependent Chemoresistance in T-ALL
Damon Runyon Cancer Research Foundation
PI ($450,000)
This project aims to define the mechanism through which EZH2 inactivation induces resistance to mitochondrial apoptosis in T-ALL, and to develop a therapeutic strategy to restore chemosensitivity in EZH2-mutant T-ALL.

2015-2020 Rational Therapy of Refractory T-Cell Acute Lymphoblastic Leukemia
Boston Children’s Hospital Translational Research Program
PI ($603,900)
This award supports our efforts to develop rationally devised therapies for treatment-resistant T-ALL, and translate these findings to the clinic in innovative investigator-initiated clinical trials.

2015-2020 Discovery and Targeting of Apoptosis Resistance Mechanisms in High-Risk T-ALL
NIH/NCI 1R01CA193651
PI ($1,534,570)
This grant, which is the scientific continuation of NCI R21 CA167124, aims to 1) Define the molecular mechanism through which EZH2 inactivation induces resistance to mitochondrial apoptosis; 2) Develop an effective therapeutic strategy to reverse apoptosis resistance and restore chemosensitivity.

Report of Local Teaching and Training

Teaching of Students in Courses
2007- Hematology Pathophysiology course Harvard Medical School 2nd year medical students 6 x 2-hour sessions per year
2014 Translational Investigation of T-ALL Harvard School of Public Health Biostatistics graduate students 2-hour lecture

Clinical Supervisory and Training Responsibilities
2007- Preceptor for hematology/oncology fellows in pediatric oncology clinic  
1 day per month
2007- Supervision of fellows, residents and medical students as attending physician on the inpatient hematology or oncology services at Boston Children’s Hospital  
4 weeks (160 hrs) /year
2012-2014 Invited Faculty Discussant, Senior Pediatric Resident Rounds, Boston Children’s Hospital  
10 hours/year

**Formally Supervised Trainees**

2012 Research advisor for Kim Bodaar, a medical student who performed her Masters thesis in my laboratory as part of the Honors Program in Medical Sciences at Radboud University, Nijmegen, The Netherlands.

2013- Postdoctoral Research Advisor for Melissa Burns MD, Fellow in Pediatric Hematology/Oncology, Boston Children’s Hospital and Dana-Farber Cancer Institute.

2013- Postdoctoral Research Advisor for Gayle Pouliot MD PhD, Fellow in Pediatric Hematology/Oncology, Boston Children’s Hospital and Dana-Farber Cancer Institute.

2014- Postdoctoral Research Advisor for Ingrid Aries PhD, Postdoctoral Research Fellow, Boston Children’s Hospital.

2015 Research advisor for Mina Jacob, a medical student performing his Masters thesis in my lab as part of the Honors Program in Medical Sciences at Radboud University, Nijmegen, The Netherlands

2015- Postdoctoral Research Advisor for Sarah Morton MD PhD, Neonatology Fellow, Boston Children’s Hospital

**Local Invited Presentations**

*Those presentations sponsored by outside entities are so noted and the sponsors are identified below.*

2011 International Outreach in Pediatric Oncology/International Medicine Grand Rounds  
Boston Children’s Hospital
2011 Translational Investigation of Molecular Oncogenesis Using Cancer Genomics and Zebrafish Models/Invited Lecture  
Boston Children’s Hospital /Dana-Farber Cancer Institute
2013 Models for Program Building in International Oncology/Panelist  
Global Oncology Seminar, Dana-Farber Cancer Institute/Harvard Medical School
2013 Pathobiology of High-Risk T-Cell Acute Lymphoblastic Leukemia  
Hematopoietic Stem Cell Transplantation Seminar, Dana-Farber Cancer Institute
2014 Translational Investigation of High-Risk T-cell Acute Lymphoblastic Leukemia  
Pediatric Grand Rounds, Boston Children’s Hospital
2014 Prognostic Biomarkers and Novel Therapies for High-Risk T-ALL  
Adult BMT Grand Rounds, Dana-Farber Cancer Institute
2014 Therapeutic PP2A Activation for High-Risk T-ALL  
Leukemia Basic and Translational Research Meeting, Dana-Farber/Harvard Cancer Center
2014 High-Risk T-Cell Acute Lymphoblastic Leukemia  
Pediatric Hematology/Oncology Research Seminar Series, Dana-Farber/Boston Children’s Hospital
Report of Regional, National and International Invited Teaching and Presentations

Invited Presentations and Courses

Regional

Those presentations sponsored by outside entities are so noted and the sponsors are identified below.

2012  Careers in Basic and Clinical Research
      Workshop presentation, Latino Medical Student Association National Meeting
      Boston, MA

2015  Pathobiology of Refractory T-cell Acute Lymphoblastic Leukemia
      University of Massachusetts Medical School
      Worcester, MA

2015  High-Risk T-cell Acute Lymphoblastic Leukemia
      Hematology/Oncology Grand Rounds, Boston Medical Center
      Boston, MA

2015  Discovery and Targeting Chemotherapy Resistance Mechanisms in High-Risk T-ALL
      Pediatric Grand Rounds, Hasbro Children’s Hospital/Brown University
      Providence, RI

National

Those presentations sponsored by outside entities are so noted and the sponsors are identified below.

2009  Absence of T-Cell Receptor Gene Rearrangements Predicts Induction Failure in Pediatric
      T-Cell Acute Lymphoblastic Leukemia/Platform Presentation (abstract)
      American Society of Hematology Annual Meeting Oral Presentation
      New Orleans, LA

2010  Pten Inactivation Promotes Loss of MYC Oncogene Addiction in a Conditional Zebrafish
      Model of T-ALL/Plenary Presentation-Young Investigator Award (abstract)
      American Society of Pediatric Hematology-Oncology 23rd Annual Meeting
      Montreal, Canada

2010  Symposium on Pediatric Oncology in Developing Countries/Panelist
      American Society of Pediatric Hematology-Oncology 23rd Annual Meeting
      Montreal, Canada

2011  Genomics and Zebrafish Models of High-Risk Leukemias and Sarcomas/Invited Lecture
      The Children’s Hospital of Philadelphia

2011  Discovery of Molecular Oncogenesis and Therapeutic Targets using Cancer Genomics and
      Zebrafish Models /Invited Lecture
      New York University Cancer Institute

2011  Translational Investigation of Molecular Oncogenesis Using Cancer Genomics and
      Zebrafish Models/Invited Lecture
      St. Jude Children’s Research Hospital

2011  Zebrafish Models of High-Risk Cancers for Discovery of Novel Therapeutic
      Strategies/Invited lecture
      University of Texas Southwestern Medical Center

2011  Genomics and Zebrafish Models of High-Risk T-ALL/Grand Rounds
UCSF Benioff Children’s Hospital

2014
Targeting chemotherapy resistance mechanisms in high-risk T-ALL/Invited Lecture
University Hospitals Case Medical Center, Cleveland, OH

2015
**Phenotypic Screening to Optimize Cancer Therapy/Invited Lecture**
AACR Annual Meeting. **Sponsored by the American Association for Cancer Research. Philadelphia, PA.**

2015
Chemotherapy Resistance Mechanisms in High-Risk T-ALL/Platform Presentation
Children’s Oncology Group Annual Meeting. **Sponsored by the Children’s Oncology Group.**

2016
Genetic Basis of Chemotherapy Resistance in T-ALL/Invited Lecture
NIH/NHGRI, Bethesda, MD

**International**

*Those presentations sponsored by outside entities are so noted and the sponsors are identified below.*

2008
Conditional Induction of T-ALL using Estrogen Receptor Fusion and Cre/Lox Approaches
Invited Oral Presentation, Targeting & Conditional Gene Expression Workshop
8th International Meeting on Zebrafish Development & Genetics. **Sponsored by the Genetics Society of America Conferences.**
Madison, WI

2010
Pten Inactivation Promotes Loss of MYC Oncogene Addiction in a Conditional Zebrafish Model of T-ALL/Platform Presentation (abstract)
European Hematology Association Scientific Workshop: T-Cell Acute Lymphoblastic Leukemia Meets Normal T-Cell Development. **Sponsored by the European Hematology Association.**
Mandelieu, France

2010
Absence of T-Cell Receptor Gene Rearrangements Predicts Induction Failure in Pediatric T-Cell Acute Lymphoblastic Leukemia/Platform Presentation (abstract)
Societe Internationale d’Oncologie Pediatrique (SIOP) Annual Meeting. **Sponsored by the SIOP professional society.**
Boston, MA

2010
A Twinning Program: The Colombian National Cancer Institute and Dana-Farber/Children’s Hospital Cancer Center/Platform Presentation
International Society of Paediatric Oncology (SIOP) Annual Meeting
Boston, MA

2013
Zebrafish Chemical Genetics for Discovery of Novel T-ALL Therapeutics/Invited Lecture
Ghent University, Belgium

2013
**Dissecting the Pathobiology of Well-Differentiated Liposarcoma using the Zebrafish Model System/Platform Presentation**
Third International Conference on Sarcoma Biology, New York, NY

2014
Perphenazine induces apoptosis in T-ALL by direct activation of PP2A/Platform Presentation (Abstract)
FASEB Research Conference: Protein Phosphatases, Nassau, Bahamas. **Sponsored by the Federation of American Societies for Experimental Biology**

**Report of Clinical Activities and Innovations**

**Current Licensure and Certification**

2004-
Massachusetts Medical License
2004-2011 General Pediatrics Board Certification
2009- Pediatric Hematology-Oncology Subspecialty Board Certification

Practice Activities
2007- Ambulatory care Pediatric oncology, DFCI 2 sessions per month
2007-2009 Inpatient attending Pediatric hematology, BCH 4 weeks per year
2009- Inpatient attending Pediatric oncology, BCH 4 weeks per year

Report of Education of Patients and Service to the Community
Activities
2008-2015 International outreach program in pediatric oncology between DFCI/BCH and Instituto Nacional de Cancerologia, Bogota, Colombia
I developed an international outreach program between our DFCI/BCH program and the pediatric oncology program of a public hospital caring for the poor in Bogota, Colombia. The goal of this program is to improve cancer care for poor children in Colombia. This program was supported by an award from World Child Cancer foundation in the UK.

Report of Scholarship

Publications

Peer reviewed publications in print or other media

Scientific Publications


**See Research Watch, Cancer Discovery 2014;4:OF14.**


Non-peer reviewed scientific or medical publications/materials in print or other media

Reviews, Chapters, and Editorials


Case Reports


Abstracts, Poster Presentations and Exhibits Presented atProfessional Meetings

Narrative Report
I am a pediatric oncology physician-scientist who spends 85% of my time and effort leading an
independent research program focused on the molecular pathogenesis of high-risk T-cell acute lymphoblastic leukemia. Our approach combines human cancer genomics with functional genetics, biochemistry and small molecule screens in human cells and in the zebrafish model system. During my postdoctoral studies, I discovered the first biomarker of failure of induction chemotherapy in human T-ALL, and our work is focused on developing novel effective therapies for these patients. We have found that EZH2 loss-of-function mutations induce resistance to conventional chemotherapy by inhibiting mitochondrial apoptosis induction, and current efforts are focused on defining the molecular mechanism linking EZH2 to the mitochondrial apoptotic machinery. We also used a zebrafish screen coupled with a novel proteomics approach to discover that perphenazine, an FDA-approved antipsychotic, effectively kills T-ALL cells by directly activating the phosphatase activity of the PP2A tumor suppressor. Current efforts are focused on investigating the structural basis of PP2A activation by perphenazine, defining the mechanism through which PP2A activation kills T-ALL cells, and screening for more potent and selective PP2A activators lacking the off-target toxicity of perphenazine. The development of specific PP2A activators could improve clinical therapy for the broad range of human cancers driven by hyper-phosphorylated PP2A substrates.

In addition to my basic and translational research activities, I also spend 15% time and effort caring for children with cancer and blood disorders through my clinical work in pediatric hematology/oncology at Boston Children’s Hospital and Dana-Farber Cancer Institute. I am involved in the supervision and teaching of pediatric hematology/oncology fellows, pediatric residents, and medical students, and I am a tutorial session leader in the HMS hematology course for second year medical and dental students. I am the postdoctoral research advisor of Melissa Burns MD and Gayle Pouliot MD PhD (both Hematology/Oncology Fellows at Boston Children’s Hospital/Dana-Farber Cancer Institute), Ingrid Aries PhD, a Postdoctoral Research Fellow in my laboratory, and Sarah Morton MD PhD (Neonatology Fellow at Boston Children’s Hospital).