Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Fox Chase Cancer Center

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 – 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Maria Gill, M.B.A.

4. **Grant Contact Person's Telephone Number:** 215-728-2659

5. **Grant SAP Number:** 4100050895

6. **Project Number and Title of Research Project:** 4 - Role of a Novel Zinc Finger Factor in Hematopoietic Development

7. **Start and End Date of Research Project:** 1/1/2010 – 12/31/2011

8. **Name of Principal Investigator for the Research Project:** Jennifer Rhodes, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $645,048

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Rhodes, Jennifer	Principal Investigator	30% Years 1-2	$68,595.32
Datta, Madhusmita	Scientific Technician	100% Year 1	$50,436.88
Bilbee, Alison	Scientific Technician	100% Years 1-2	$72,736.54
Robu, Mara	Postdoctoral Associate	42% Year 2	$27,637.97
McDaniels, Suzanne	Summer Assistant	100% Years 1-2	$4,375.21
Ulrich, Allison	Scientific Technician	100% Year 2	$44,086.48

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Dissecting the oncogenic and molecular function of a novel hematopoietic zinc finger factor	☐NIH ☐ Other federal (specify:_____ _____) ☒ Nonfederal source (specify: V Foundation)	August 2010	$200,000	$200,000
Mechanism of Drl-L3 Mediated Hematopoietic Progenitor Cell Differentiation	☐NIH ☐ Other federal (specify:_____ _____) ☒ Nonfederal source (specify: American Cancer Society)	October 2013	$778,254	pending
Role of Zinc Finger Factor Drl.3 in Hematopoietic Differentiation	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	February 2013	$2,231,250	Not funded

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

A grant expanding on the CURE funded research is under review at the American Cancer Society. The NIH applications did not receive funding.

12. Future of Research Project. What are the future plans for this research project?

To better understand the G4-controlled molecular pathways that are important in healthy and malignant hematopoiesis and to pursue additional funding for these efforts.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female	1			1
Unknown				
Total	**1**			**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1			1
Unknown				
Total	**1**			**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			1
Black				
Asian				
Other				
Unknown				
Total	**1**			**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No___X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. <u>Provide detailed results of the project.</u> Include

evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Research Project 4: Project Goals, Objectives/Specific Aims and Progress

Goals

Role of a Novel Zinc Finger Factor in Hematopoietic Development - The purpose of this project is to understand the role of a novel erythroid factor (G4) in hematopoietic development using the zebrafish model system. Since the molecular basis for G4 function in controlling erythropoiesis is completely unknown, we will determine where *g4* fits in the hierarchy of transcription factors controlling hematopoietic development, identify its human ortholog, and perform structure/function analysis to identify the domains of *g4* that are essential for its function. Results from these studies will provide insights into how normal blood cell development is controlled and how those processes are perturbed in cancer.

Objectives and Aims

A network of transcriptional regulators coordinates the cell fate choices and progressive differentiation of hematopoietic stem cells, a process that is incompletely understood. Hematopoiesis is conserved throughout vertebrate evolution, and we have employed the facile zebrafish experimental model system to identify a novel molecular effector, called *g4*, that is critical for erythroid development. *G4* is part of a cluster of highly homologous genes in zebrafish, and the corresponding human gene cluster encodes zinc finger factors of unknown function. The objective of this project is to investigate the role of *g4* in hematopoietic development using the zebrafish model system. We intend to do so according to the following aims:

Aim 1. Use epistasis analysis to determine where g4 function is positioned within the hierarchy of known regulators of erythroid development. This will be accomplished by performing morpholino knockdown of known molecular effectors of primitive hematopoiesis (*g4*, *scl*, *gata1*, *pu.1*), determining how this affects *g4* expression, and assessing whether forced expression of *g4* mRNA rescues the developmental blockade.

Aim 2. Utilize a complementation strategy to investigate the specificity of function among g4 family members and identify human paralogs. We will knock down *g4* expression and determine if forced expression of the other members of the zebrafish gene cluster or the human paralogs are able to restore development. Because the zebrafish family members are highly similar, this may inform the structure/function analysis if differences among family members are identified.

Aim 3. Perform structure/function analysis to identify domains or motifs of g4 that are essential for function. We will do so by determining if mutant *g4* constructs lacking conserved domains or motifs are able to rescue the developmental arrest caused by *g4* knockdown.

Together, these studies will provide insight into the function of g4 in normal development of erythroid precursors as well as to how its misregulation might contribute to their transformation.

Final Progress Report

Aim 1. Use epistasis analysis to determine where g4 function is positioned within the hierarchy of known regulators of erythroid development.

Mammalian and zebrafish models have been used to establish essential roles for *pu.1* in white blood cell development and *gata1* in red cell development, defining a cross-antagonistic function for these factors in determining hematopoietic progenitor cell fate. This is evidenced by morpholino knockdown of zebrafish *gata1* which blocks hematopoietic progenitor cell differentiation to the erythroid lineage and instead causes the progenitor cells to transfate to the myeloid lineage. Similarly, *pu.1* morpholino knockdown causes a loss of myeloid cells and transfates the progenitor cells to the erythroid lineage. Since erythropoiesis occurs exclusively in the posterior hematopoietic region in the zebrafish embryo, while myelopoiesis occurs in both the posterior and anterior hematopoietic compartments, *pu.1* morpholino-injected (morphant) embryos display ectopic erythroid development in the anterior of the embryo. Our studies focused on the interplay of the novel gene *g4* with the *pu.1-gata1* axis regulating hematopoiesis.

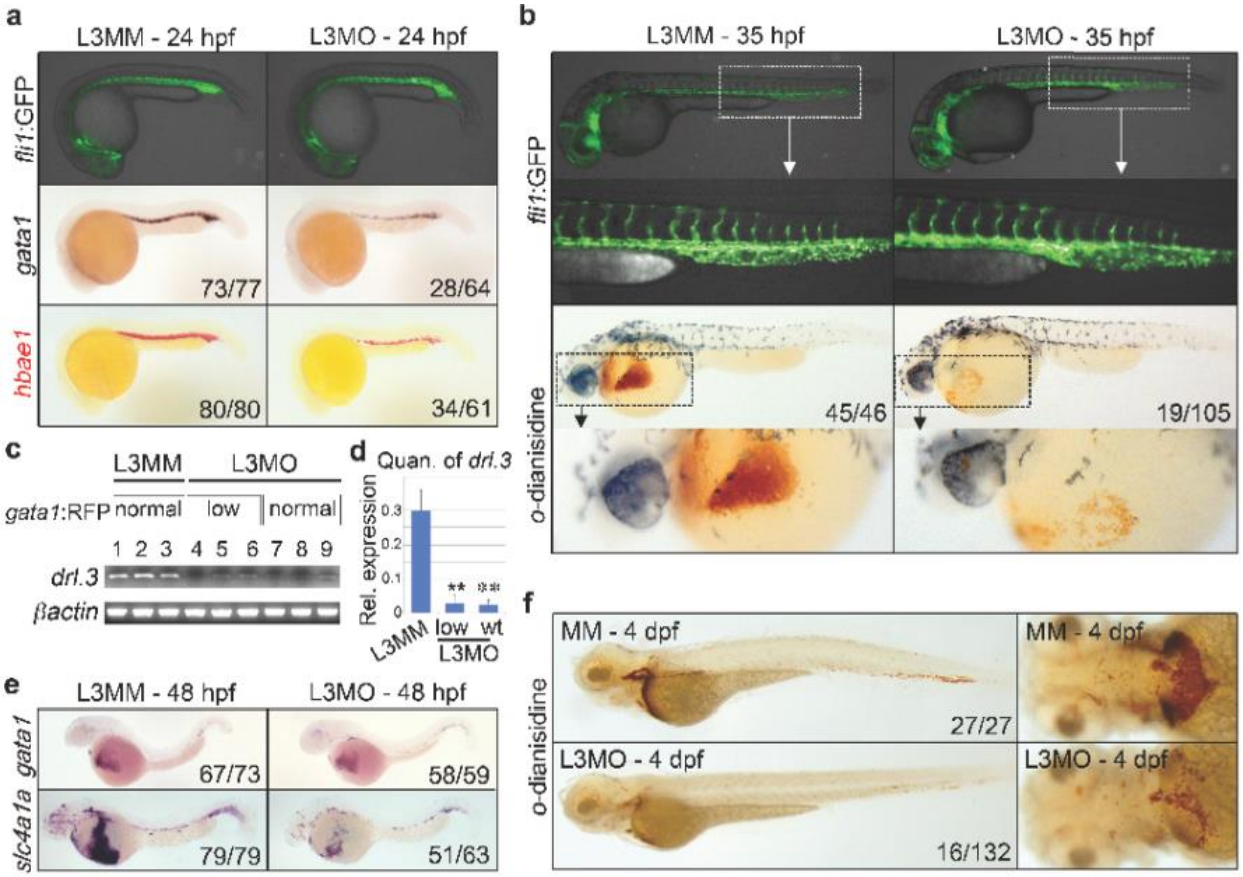


Figure 1. G4/*Drl.3* is essential for erythroid development.
(a) Control- (mismatch, L3MM) and *drl.3* morpholino- (L3MO) injected embryos at 24 hpf showing live Tg*(fli1a:EGFP)* marking of the vasculature and *gata1* and *hbae1* WISH, as indicated. (b) Embryos at 35 hpf showing live Tg*(fli1a:EGFP)* pattern, with a magnified view of the PBI region, and *o*-dianisidine stained embryos with an enlargement of the anterior region. (c) RT-PCR analysis of individual Tg*(gata1:DsRED)* control and L3MO-injected embryos. Drl.3 morphants were sorted for normal or decreased (estimated $\leq$ 60% of normal) numbers of circulating erythrocytes at 48 hpf. (d) Quantitation of the levels of *drl.3* expression normalized to *b-actin* from RT-PCR analysis in (c). $^{**}P = 0.0019$ and $^{**}P = 0.0015$ (Student's t-test). (e) WISH of *gata1* and *slc4a1a* in 48 hpf control and *drl.3* morphants. (f) *O*-dianisidine stained control and *drl.3* morphants at 4 dpf; lateral views (left panels) and ventral views of the anterior region (right panels). (a-b, e-f) The number of the embryos with the representative phenotype per total number of embryos is shown; lateral views with head to the left, dorsal upward.

To examine whether *g4* is essential for erythroid development, we examined the expression pattern of *gata1* in *g4* morphant embryos. We have found a decreased number of cells expressing *gata1* in *g4* deficient embryos as determined by whole mount RNA in situ analysis (Figure 1a) at 24 hours post-fertilization (hpf). G4 is not necessary for the normal development of the vasculature as determined by a normal pattern of *fli1*:GFP transgene expression in *g4* morphants (Figure 1a-b). The incomplete penetrance of the phenotype was not due to the efficacy of *g4* knockdown, as affected and unaffected embryos had similar levels of wild-type *g4* message, which was depleted but not completely absent (Figure 1c). While *gata1* expression appeared normal at 48 hpf, markers of more mature erythrocytes were decreased through 4 dpf in *g4* morphants (Figure 1e-f).

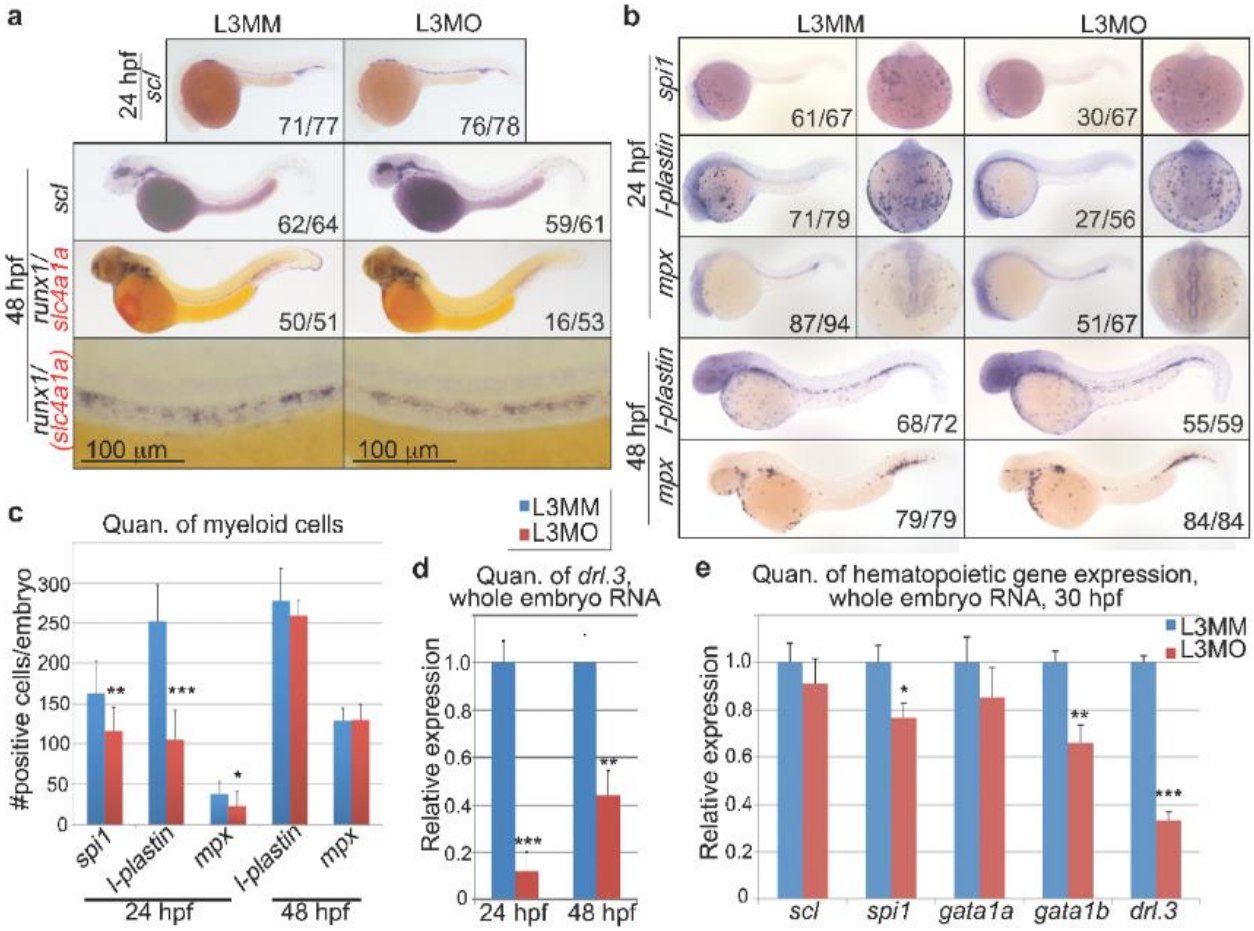


Figure 2. Knockdown of *g4*/*drl.3* transiently decreases myeloid cells without altering the emergence of primitive progenitor and definitive stem cells.

(a) WISH of *scl* at 24 and 48 hpf, and *runx1* (dark blue)/ *slc4a1a* (red) at 48 hpf in L3MM control- and L3MO-injected embryos, as labeled. Embryos shown as lateral views. (b) WISH of *spi1*, *l-plastin* and *mpx* at 24 hpf and *l-plastin* and *mpx* 48 hpf. Dorsal, anterior (24 hpf only, right panels) and lateral views are shown. (c) Quantitation of the number of the WISH *spi1*$^+$, *l-plastin*$^+$ and *mpx*$^+$ cells in the anterior of the embryo at 24 hpf and total body *l-plastin*$^+$ and *mpx*$^+$ cells at 48 hpf in control (L3MM, blue) and L3MO-injected (red) embryos (N=8 for each column except for *mpx* at 24 hpf where N=15, bars show mean ± S.E.). $^{**}P = 0.0044$, $^{***}P < 0.0001$ and $^{*}P = 0.0156$ (Student's t-test). (d) Quantitative real-time

PCR analysis of *drl.3* in whole embryo RNA samples from 24 and 48 hpf *drl.3* morphants (red) and controls (L3MM, blue, set to 1, arbitrary units). $^{**}P = 0.0042$ and $^{***}P < 0.0001$ (Student's t-test). (e) Quantitative real time PCR analysis of *scl*, *spi1*, *gata1a*, *gata1b*, and *drl.3* in whole embryo RNA samples from pools of 30 hpf *drl.3* morphants (red) compared to control-injected embryos (blue, set to 1, arbitrary units). $^{*}P = 0.0114$, $^{**}P = 0.0083$ and $^{***}P = 0.0005$ (Student's t-test). (d-e) Bars show mean ± S.D., from three independent experiments. Expression was normalized to *gapdh*.

In contrast to *gata1* morphants that show an expansion of *pu.1*-positive cells in the erythroid blood-forming region of the embryo, 24 hpf *g4* morphants have slightly decreased numbers of *pu.1*-expressing cells (Figure 2). Additionally, at 24 hpf, embryos deficient for *g4* have half the number of myeloid *l-plastin*-expressing cells compared with control-injected embryos (data not shown). These data indicate that *g4* is important for the development of both primitive erythroid and myeloid lineages. However, 48 hpf *g4* morphants display normal numbers of myeloid cells and runx1^{+} stem/progenitor cells in the aorta-gonad-mesonephros region (Figure 2).

We wanted to examine the functional overlap between *gata1* and *g4* because the *g4* morphant does not mimic the *gata1* morphant. The erythroid and myeloid defects in *g4*-deficient embryos (morphants) could be reverted back to a wild-type state by co-injecting *g4* mRNA with the *g4* morpholino (Figure 3). However, enforced expression of *gata1* was <u>not</u> sufficient to rescue *g4* morphants (Figure 4). Furthermore, we found that enforced expression of *g4* is <u>not</u> sufficient to rescue the erythroid deficiency in *gata1*-depleted embryos (Figure 4). Likewise, depletion of *g4* leads to decreased expression of *g4* and *gata1* (Figure 5 and Figure 4). We performed morpholino-based studies to show that *gata1* knockdown resulted in a severe reduction in cells expressing *g4* while *pu.1* knockdown caused an expansion of *g4* expression in the anterior myeloid compartment (Figure 4). These data are consistent with *g4* expression tracking closely with the erythroid lineage.

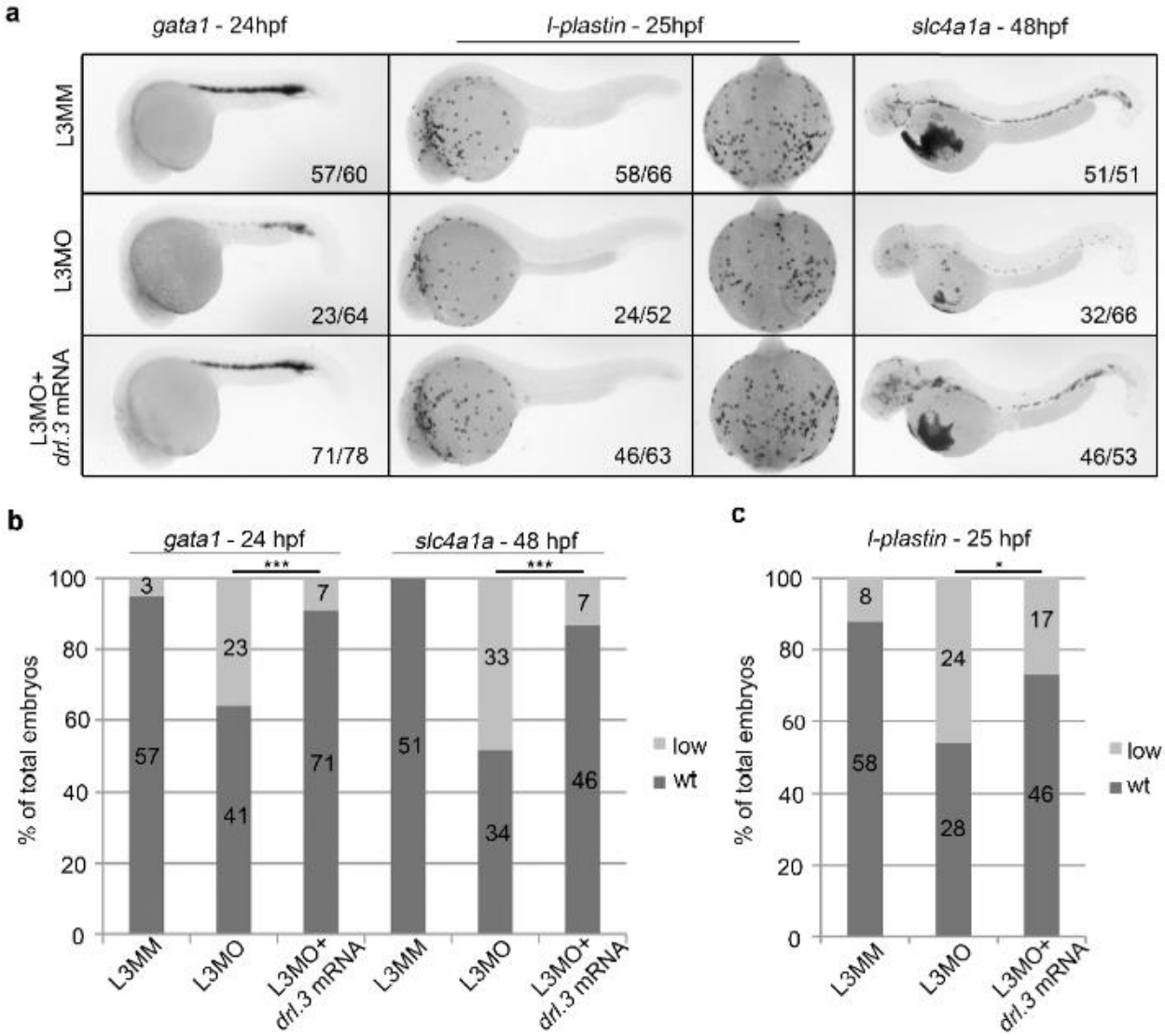


Figure 3. Morpholino-induced defects are due to knockdown of *g4*/*drl.3* activity.
(a) WISH of *gata1* at 24 hpf, *l-plastin* at 25 hpf, and *slc4a1a* at 48 hpf in L3MM- and L3MO-injected embryos and embryos co-injected with L3MO and *drl.3* mRNA. Embryos shown as lateral views, head left. WISH for *l-plastin* also shows dorsal, anterior views. (b) Percent of L3MM- and L3MO-injected and L3MO/*drl.3* mRNA co-injected embryos with normal (wt) or decreased (*; estimated ≤ 60% of normal) numbers of *gata1* or *slc4a1a*-expressing cells. The numbers of embryos are indicated in the columns. ***$P < 0.0001$ (Fisher's exact test). (c) Percent of embryos with normal (wt) or decreased (*) numbers of *l-plastin*-expressing cells, as labeled. The numbers of embryos are indicated in the columns. *$P = 0.0445$ (Fisher's exact test).

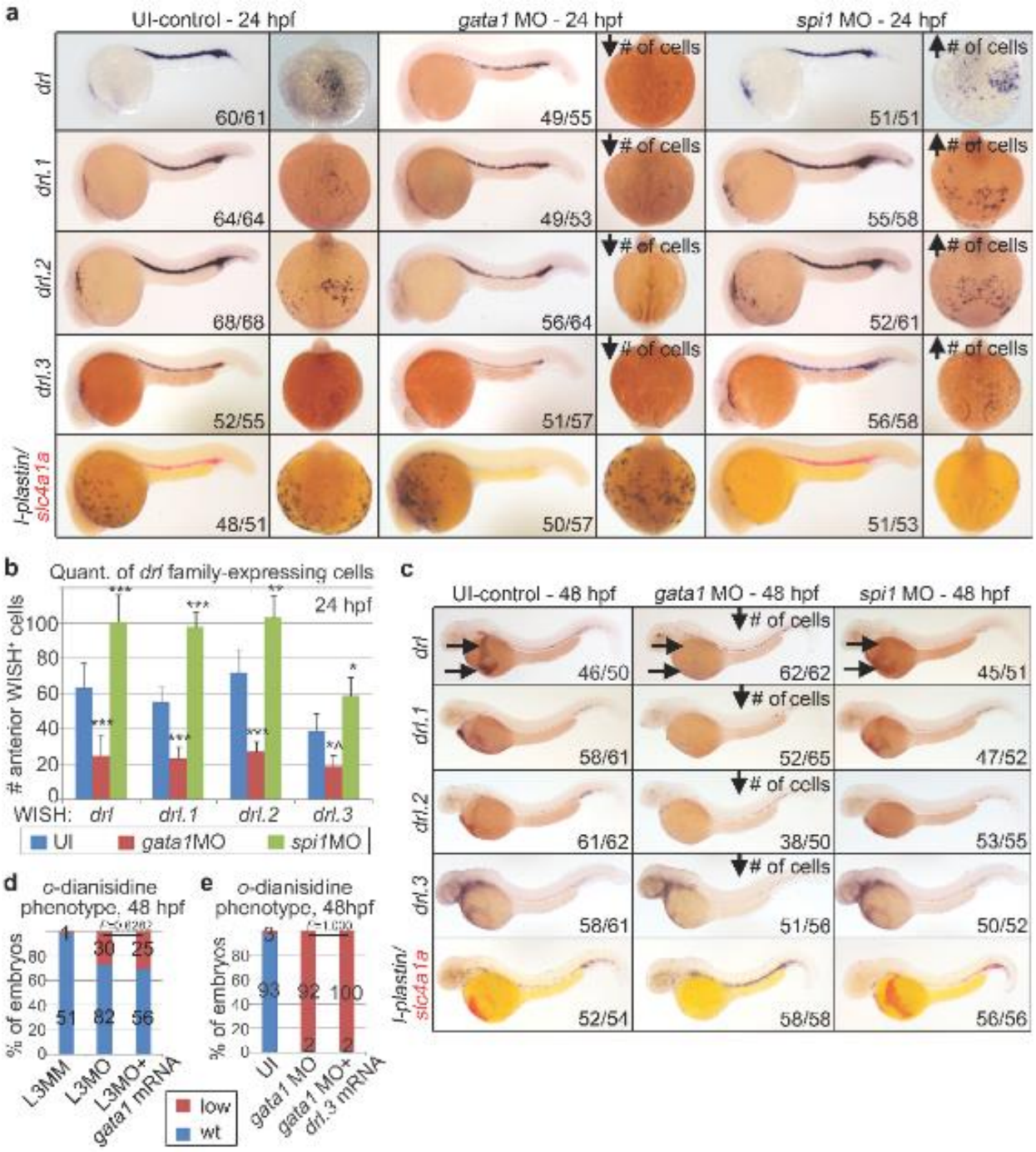


Figure 4. Loss of *gata1* and *spi1* affect *g4/drl* gene family expression.
(a) WISH of *drl* family members in *gata1* and *spi1* morphants (MO) compared to uninjected embryos at 24 hpf. From top to bottom: *drl*, *drl.1*, *drl.2*, *drl.3*, and *l-plastin* (dark blue)/*slc4a1a* (red). Lateral views, head to the left (left panels); anterior, dorsal views (right panels). The number of the embryos with the representative phenotype out of the total number of embryos is indicated. Arrows in the panels showing dorsal views indicate an increase or decrease in the numbers of WISH$^+$ cells. (b) Quantitation of the number of *drl* gene family-expressing cells in the anterior hematopoietic region of control (UI), *gata1* morphants and *spi1* morphants at 24 hpf. N=10 for each column. Bar shows mean ± S.D. **P* = 0.0013, ***P* = 0.0002, *^*P* = 0.0003 and ****P* ≤ 0.0001 (Student's t-test). (c) WISH of *drl* family members in the

indicated embryos at 48 hpf. Embryos shown as lateral views. Horizontal arrows indicate the region where cells in circulation can be visualized. Downward facing arrows indicate decreased $WISH^+$ cell numbers. (d) Percent of L3MM-injected, L3MO-injected, and L3MO/*gata1* mRNA co-injected embryos that have normal (blue, wt) or low numbers of erythroid cells (red, *estimated ≤ 60% of normal) based on *o*-dianisidine staining at 48 hpf. (e) Quantitative analysis of uninjected, *gata1* MO-injected, and *gata1* MO/*drl.3* mRNA co-injected embryos that have normal (blue, wt) or low numbers of erythroid cells (red, *) based on *o*-dianisidine staining at 48 hpf. (d-e) Numbers of embryos are indicated in the columns. Statistical significance was analyzed using Fisher's exact test.

In sum, these data indicate that *gata1* and *g4* do not participate in a linear pathway and suggest that these factors have functions in addition to supporting the expression of each other. Possible explanations are that the stem/progenitor cells are impaired from further differentiation or/and the cells do not survive. The stem cell marker *scl* was present at comparable levels in control and *g4*-deficient embryos (data not shown), indicating that the *g4*-deficiency does not inhibit formation of a stem/progenitor cell population. In total, our data indicate that g4 is a new, important regulator of primitive hematopoiesis in zebrafish.

Aim 2. Utilize a complementation strategy to investigate the specificity of function among g4 family members and identify human paralogs.

To address this aim we planned to knock down *g4* and determine if forced expression of the other members of the zebrafish gene cluster or the human paralogs are able to restore development. Indeed, depletion of *g4* leads to decreased expression of the other gene family members; however, enforced expression of the founding member of this family did <u>not</u> rescue the erythroid or myeloid cell deficiencies in *g4* morphants (Figure 5). This indicates that *g4* has a specific and essential role in hematopoiesis. This also indicates that the three regions of G4 that are not homologous to the other gene family members have important roles in the function of the protein. The preliminary data generated from Aims 1 and 2 have been submitted to the journal *Scientific Reports* to be considered for publication and are the basis for an American Cancer Society Research Scholar Award application.

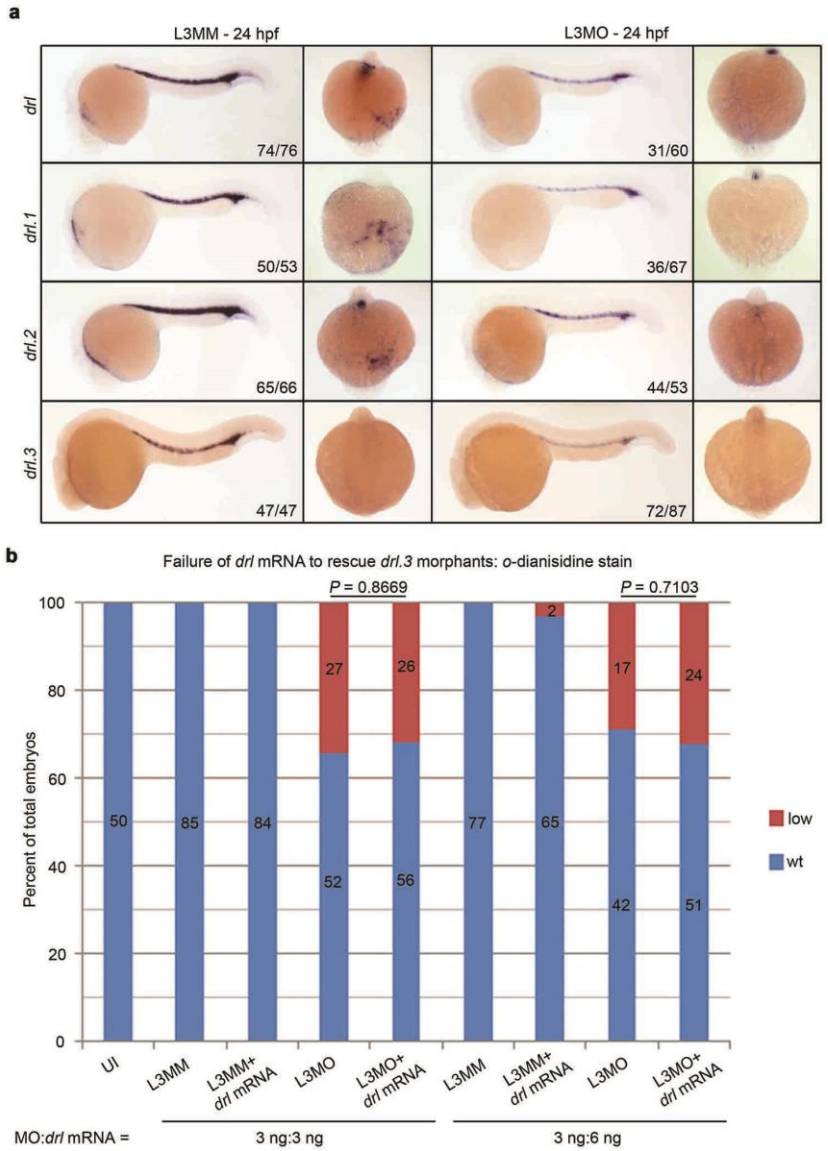


Figure 5. Enforced expression of *gfamily/drl* is not sufficient to rescue *g4/drl.3* morphants. (a) WISH of drl, drl.1, drl.2, and drl.3 in 24 hpf L3MM- and L3MO-injected embryos. Lateral views, head to the left (right) and dorsal anterior views (left). Number of embryos with representative phenotypes per total embryos analyzed is indicated. (b) Percent of control (L3MM) or drl.3 morphants (L3MO) ± drl mRNA that display normal (wt) or low (estimated ≤ 60% of normal) numbers of o-dianisidine+ cells. Two amounts of drl mRNA were tested: 3 ng/embryo and 6 ng/embryo. Co-injection of L3MO + 3 ng of drl mRNA per embryo resulted in 32% of embryos showing decreased numbers of o-dianisidine+ cells versus 34% of drl.3 morphants. Similarly, 6 ng drl mRNA + L3MO resulted in 32% of the embryos displaying decreased erythrocytes compared to 29% of the L3MO-injected morphants. P values determined by Fisher's exact test.

Next, we turned our focus to determining whether the potential human homologs are functionally equivalent to *g4* in zebrafish. Homology, synteny and phylogeny did not identify an obvious candidate (Figure 6). To narrow which human genes are more likely to be homologs of *g4*, we consulted with experts in the FCCC Bioinformatics and Molecular Modeling core facilities. Zebrafish G4 contains multiple consecutive Cys_2-His_2 (C2H2) zinc-finger domains and the gene is strongly expressed in embryonic hematopoietic stem and progenitor cells, but expression is barely detectable in mature myeloid cells. We used bioinformatics to identify human genes that had similar patterns of expression and were homologous to G4 protein. We identified 15 putative human *g4* homologs. Synteny, homology, and expression analyses did not make obvious which one of these genes is most likely to be the *g4* homolog. Oncomine expression data indicates that many of the putative *g4* human homologs have aberrant expression levels in a variety of leukemias, although expression levels were often normal in pre-leukemic (MDS) samples. The pathogenic contributions of the putative *g4* homologs have not been examined. Future studies will examine the function of the newly identified, putative human *g4* homologs.

Figure 6. (Below) G4/Drl.3 cross-species homology and synteny analyses.
(a) Analysis of synteny of the zebrafish *drl* genomic region compared to human, mouse and chicken genomes. (b) Comparison of human gene region on Chr. 17 encompassing *PEX12* and *AP2B1*, which flank the *g4/drl* family in zebrafish, to the genomic locations of the equivalent genes in the zebrafish, mouse, and chicken genomes. X=no homolog identified. (c) Genomic locations of human genes encoding proteins homologous to Drl.3. (d) Chromosomal locations of the human genes encoding Drl.3-like proteins and the murine genes encoding proteins that are similar to these human proteins. Murine genes were identified by homolog name (Murine Gene Accession, 3rd column) or the translated human proteins were used as query sequences into NCBI murine protein databases as indicated (columns 5-8). Top two hits are shown. Genes are listed by gene name and, if available, accession number. Chromosomal locations are listed to the right of the genes. ND=not found. Note the high homology of several mouse proteins to different human factors (BLASTp: Zfp27, EDL39830.1, EDL10295.1, etc.).

a Synteny of zebrafish genes flanking the *drl* cluster

Danio rerio Chr. 5 Genes	Chromosomal Location of Homologs		
	Homo sapiens	Mus musculus	Gallus gallus
taf6	7q22.1	5	Un
zfpl1	11q13	5	x
scarb2	4q21.1	5	c4
doc2b	17p13.3	11	c19
gats12	x	x	x
wbscr16	7q11.23	5	c19
ncf1	7q11.23	5	c19
polr2j	7q22.1	5	x
lrwd1	7q22.1	5	c19
bckdk	16p11.2	7	x
pex12	17q12	11	c19
drl.3			
drl.2			
drl.1			
drl			
ap2b1	17q11.2-q12	11 B5	c19
Predicted: gas2L2	17q12	11 C	c19
LOC100537137: znf541-like	19q13.33	7 A2	x
LOC567055: ehd2	19q13.3	7	x
sepw1	19q13.3	7 A2	Un
akap10	17p11.1	11 B2	c19
aspa	17p13.3	11	c19
zswim7	17p12	11	c19
adora2b	17p12	11	c19
specc1	17p11.2	11	c19

b Synteny of human genes flanking *PEX12* and *AP2B1*

Homo sapiens Chr17 Genes	Chromosomal Location of Homologs		
	Danio rerio	Mus musculus	Gallus gallus
RAD51D	5	11	19
FNDC8	none	11	x
NLE1	10	11	19
UNC45B	8	11	19
SLC35G3	x	11	x
SLFN5	x	11	x
SLFN11	x	x	x
SLFN12	x	x	x
SLFN13	x	x	x
SLFN12L	x	x	x
SLFN14	x	11	x
SNORD7	x	11	x
PEX12	5	11	19
AP2B1	5	11	19
RASL10B	5	11	19
GAS2L2	5	11	19
MMP28	15	11	19
TAF15	15	11	19
CCL5	x	11	19
RDM1	3	11	27
LYZL6	x	11	x
CCL16	x	x	x
CCL14	x	x	x
CCL15	x	x	x

c Human genes encoding proteins homologous to Drl.3

Description	Accession	Chr. Location
zinc finger protein 160 [Homo sapiens]	NM_033288.3	19q13
zinc finger protein 234 [Homo sapiens]	NM_006630.2	19q13
zinc finger protein 665 [Homo sapiens]	NM_024733.3	19q13
zinc finger protein 585B [Homo sapiens]	NM_152279.3	19q13
zinc finger protein 624 [Homo sapiens]	NM_020787.3	17p11
zinc finger protein 347 isoform a [Homo sapiens]	NM_001172674.1	19q13
zinc finger protein 99 [Homo sapiens]	NM_001080409.2	19p12
zinc finger protein 616 [Homo sapiens]	NM_178523.3	19q13
zinc finger protein 845 [Homo sapiens]	NM_138374.1	19q13
zinc finger protein 709 [Homo sapiens]	NM_152601.3	19p13
zinc finger protein 585A [Homo sapiens]	NM_152655.2	19q13
zinc finger protein 62 homolog isoform 1 [Homo sapiens]	NM_152283.4	5q35
zinc finger protein 729 [Homo sapiens]	NM_001242680.1	19p12
zinc finger protein 226 isoform a [Homo sapiens]	NM_001032372.1	19q13
zinc finger protein 91 [Homo sapiens]	NM_003430.2	19p12
zinc finger protein 569 [Homo sapiens]	NM_152484.2	19q13
zinc finger protein 268 isoform c [Homo sapiens]	NM_001165882.2	12q24
zinc finger protein 268 isoform a [Homo sapiens]	NM_003415.2	12q24
zinc finger protein 658 [Homo sapiens]	NM_033160.5	9p13
zinc finger protein 208 [Homo sapiens]	NM_007153.3	19p12
zinc finger protein 184 [Homo sapiens]	NM_007149.2	6p21
zinc finger protein 227 [Homo sapiens]	NM_182490.1	19q13
PREDICTED: zinc finger protein 84-like [Homo sapiens]	XM_003960030.1	Un
zinc finger protein 43 isoform 1 [Homo sapiens]	NM_001256649.1	19p13

d Identification of candidate murine homologs to the human Drl.3-like factors based on gene names or protein homology.

Query [Homo sapien]	hChr. Loc.	Murine Gene Accession	mChr. Loc.	BLASTp into mus musculus (top 2 hits)	mChr. Loc.	Domain enhanced blast (DELTA-BLAST)	mChr. Loc.
ZNF160 NM_033288.3	19q13	Zfp160 NM_145483.2	17	Zfp160 NP_663458.2, Zfp160 AAH15291.1	17, 17	Zfp160 EDL38026.1, Zfp26 NP_035883.2	17, 9
ZNF234 NM_006630.2	19q13	ND		EDL10327.1, EDL39830.1	1, 13	Zfp569 NP_848471.2, Zfp74 EDL24049.1	7, 7
ZNF665 NM_024733.3	19q13	ND		BAE25340.1, Zfp160 NP_663458.2	17, 17	Zfp160 EDL38026.1, Zfp569 NP_848471.2	17, 7
ZNF585B NM_152279.3	19q13	ND		Zfp27 EDL24051.1, Zfp27 NP_001032796.1	7, 7	Zfp27 EDL24051.1, Zfp27 BAC25981.1	7, 7
ZNF624 NM_020787.3	17p11	ND		EDL39830.1, EDL10327.1	1, 13	Zfp26 NP_035883.2, EDL25096.1 (similar to Zfp347)	9, 9
ZNF347 NM_001172674.1	19q13	ND		Zfp160 EDL38026.1, EDL39830.1	17, 13	Zfp160 EDL38026.1, Zfp26 NP_035883.2	17, 9
ZNF99 NM_001080409.2	19p12	ND		NP_898969.2, NP_001156718.1	13, 13	Zfp26 NP_035883.2, EDL25096.1	9, 9
ZNF616 NM_178523.3	19q13	Zfp616 NM_001177570.1	11	EDL39830.1, Zfp658-like XP_003945964.1	1, 12	Zfp26 NP_035883.2, EDL25096.1	9, 9
ZNF845 NM_138374.1	19q13	ND		EDL39830.1, EDL16198.1	1, 13	NP_898969.2, CAM27169.1	13, 2
ZNF709 NM_152601.3	19p13	Zfp709 NM_145624.4	8	CAM27169.1, CAM21132.1	2, 2	CAM21132.1, Predicted Zfp700 XP_003688827.2	2, 2
ZNF585ANM_152655.2	19q13	ND		Zfp27 NP_001032796.1, BAC25981.1	7, 7	Zfp27 EDL24051.1, BAC25981.1	7, 7
ZNF729 NM_001242680.1	19p12	ND		EDL39830.1, NP_001156718.1	1, 13	Zfp208 NP_001004139.2, Zfp619 AAH80693.1	7, 7
ZNF226 NM_001032372.1	19q13	ND		Zfp235 EDL24353.1, Zfp235 NP_064325.2	7, 7	Zfp26 NP_035883.2, EDL25096.1	11, 9
ZNF91 NM_003430.2	19p12	Zfp91 NM_053009.3	19	EDL39830.1, EDL10295.1	1, 13	EDL39830.1, EDL10295.1	1, 13
ZNF569 NM_152484.2	19q13	Zfp569 NM_178384.3	7	Zfp569 NP_848471.2, Zfp74 AAH50048.1	7, 7	Zfp74 EDL24049.1, Zfp569 NP_848471.2	7, 7
ZNF268 NM_001165882.2	12q24	ND		EDL39830.1, CAM27169.1	1, 2	NP_898969.2, CAM27169.1	13, 2
ZNF208 NM_007153.3	19p12	Zfp208 NM_001035231.2	13	EDL39830.1, EDL10295.1	1, 13	EDL10295.1, NP_001156718.1	13, 13
P: ZNF84-like XM_003960030.1	12q24	ND		Zfp420 NP_766328.2, BAC32503.1	7, 7	Zfp26 NP_035883.2, EDL25096.1	11, 9
ZNF43 NM_001256649.1	19p13	ND		NP_898969.2, NP_001156718.1	13, 13	Zfp26 NP_035883.2, EDL25096.1	11, 10

Aim 3. Perform structure/function analysis to identify domains or motifs of g4 that are essential for function.

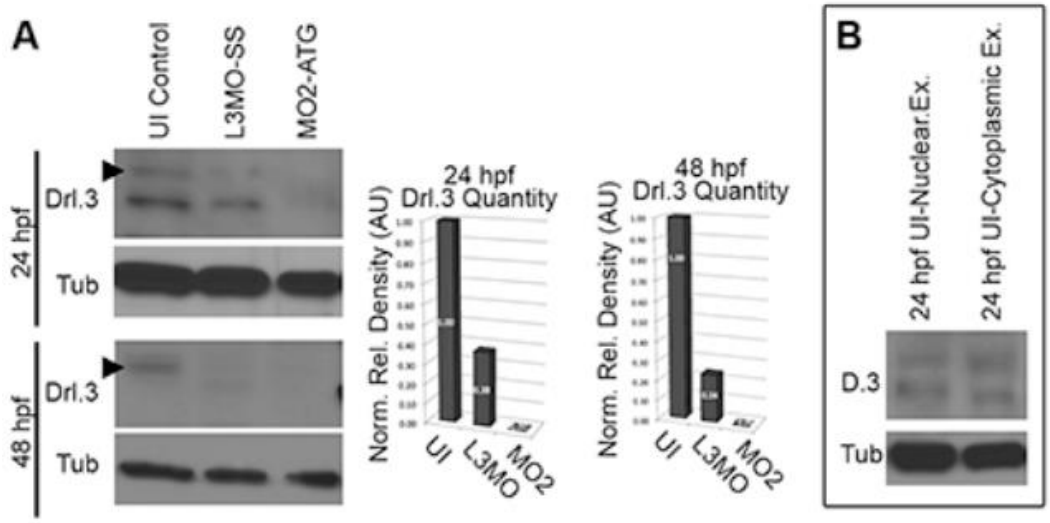
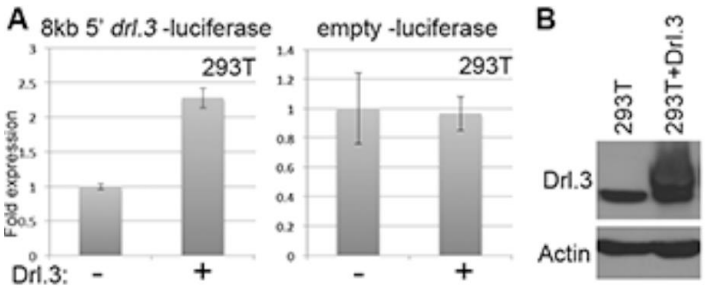


Figure 7. Detection of G4/Drl.3 protein.
(A) Western analysis of Drl.3 protein in uninjected (UI), *g4/drl.3* morphants of morphants ablated for the entire *g4* family. The protein quantity at 24 hpf and 48 hpf is shown to the right. (B) Western detection of Drl.3 protein in nuclear and cytoplasmic extracts from uninjected embryos.

Our ability to evaluate the activity of wild-type *g4* is essential for aim 3 studies. To do this, we have developed *in vitro* transcriptional reporter assays, *in vivo* rescue analysis and generated a novel antibody that recognizes the zebrafish G4 protein (Figure 7). During the grant term, we have successfully used the G4 antibody to detect the protein using western analysis of a cell line transfected to express the protein and in whole mount zebrafish embryos, although we were unsuccessful in detection endogenous protein in whole mount embryos. The initial *in vitro* transcriptional reporter assays were done using a reporter construct in which a short fragment upstream of the g4 gene was controlling expression of a luciferase reporter gene. Unfortunately, we obtained variable results using this construct. We subsequently subcloned the entire region upstream of the ATG containing exon of g4 into the luciferase reporter vector (Figure 8). Preliminary studies indicated that g4 can activate transcription from this reporter, and a more complete study is ongoing to further refine this assay (Figure 8). Thus far, these studies have shown for the first time that G4 can regulate transcription and have begun to narrow in on the upstream regulatory sequences essential for autoregulation.



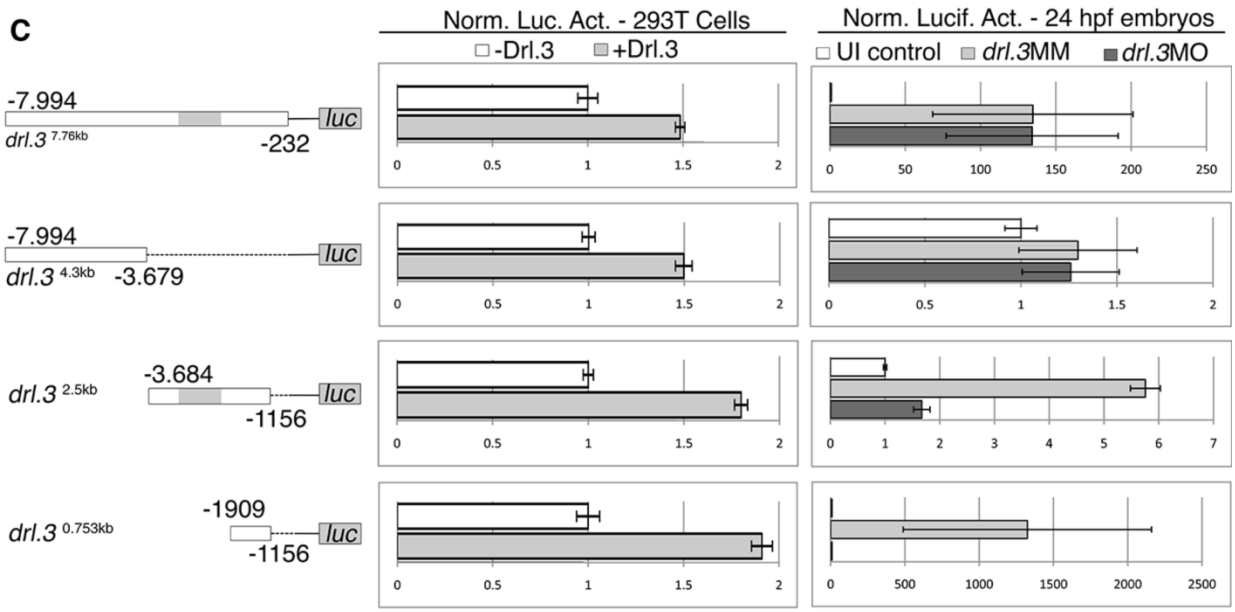


Figure 8. Autoregulatory activity of G4/Drl.3.
(A) Luciferase reporter assays showing the activation of the entire sequences upstream of the *drl.3* ATG site by Drl.3 protein in 293T cells. (B) Detection of transfected Drl.3 protein in 293T cells. (C) Luciferase reporter assay results from 293T and zebrafish embryos using the constructs diagrammed on the left.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐ Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes__X_____ No_____

If yes, please describe your plans:

We are aiming to submit the ongoing studies that stemmed from CURE funded research to peer-reviewed publications.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

See attached biosketch.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Jennifer Rhodes, Ph.D.	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) JENNIFER_RHODES	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Oregon State University, Corvallis, OR	B.S.	06/93	Biology
Oregon Health & Sciences University, Portland, OR	Ph.D.	06/00	Molecular/Med Genetics
Dana-Farber Cancer Institute, Boston, MA	Postdoc	04/00-09/04	Pediatric Oncology
Dana-Farber Cancer Institute, Boston, MA	Instructor	09/04-01/08	Pediatric Oncology

POSITIONS AND HONORS

Positions

Research Assistant, Department of Biochemistry, Oregon Health & Sciences University, Portland, OR 1993-1994

Graduate Student, Ph.D. advisor Dr. Brian J. Druker, M.D., Department of Molecular & Medical Genetics, Oregon Health & Sciences University, Portland, OR 1994-2000

Instructor, part-time, Techniques in Molecular Biology I & II (BIT 221, 223), Bioscience Technology Program, Portland Community College, Rock Creek, OR 1996-1998

Postdoctoral Fellow, laboratory of Dr. A. Thomas Look, M.D., Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA 2000-2004

Instructor, laboratory of Dr. A. Thomas Look, M.D., Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA 2004-2008

Assistant Professor, Immune Cell Development and Host Defense Program, Fox Chase Cancer Center, Philadelphia, PA 2008-date

Honors

Graduate Student Hematology Training Grant, Dr. Grover Bagby at OHSU 1996-1999

American Cancer Society, Institutional Research Grant, FCCC 2008-2009

SELECTED PEER-REVIEWED PUBLICATIONS

1. Liu, T.X., Zhou, Y., Kanki, J.P., Deng, M., **Rhodes, J.**, Yang, H.W., Sheng, X.M., Zon, L.I., Look, A.T. Evolutionary conservation of zebrafish linkage group 14 with frequently deleted regions of human chromosome 5 in myeloid malignancies. *Proc. Natl. Acad. Sci, USA.* **99**:6136-6141, 2002. PMCID: PMC122915.
2. Liu, T.X., Howlett, N.G., Deng, M., Langenau, D.M., Hsu, K., **Rhodes, J.**, Kanki, J.P., D'Andrea, A.D., Look, A.T. Knockdown of zebrafish Fancd2 causes developmental abnormalities via p53-dependent apoptosis. *Dev. Cell* **5**:903-914, 2003.
3. Hsu, K., Traver, D., Kutok, J.L., Hagen, A., Liu, T.X., Paw, B.H., **Rhodes, J.**, Berman, J.N.,

Zon, L.I., Kanki, J.P., Look, A.T. The pu.1 promoter drives myeloid gene expression in zebrafish. *Blood* **104**:1291-1297, 2004.

4. **Rhodes, J.**, Hagen, A., Hsu, K., Deng, M., Liu, T.X., Look, A.T., Kanki, J.P. Interplay of Pu1 and Gata-1 determines myelo-erythroid progenitor cell fate in zebrafish. *Dev. Cell*. **8**:97-108, 2005.
5. Liu ,T.X., **Rhodes, J.**, Deng, M., Hsu, K., Radomska, H.S., Kanki, J.P., Tenen, D.G., Look, A.T. Dominant-negative C/ebpa stimulates primitive erythropoiesis in zebrafish. *Exp. Hematol*. **35**:230-239, 2007. PMCID: PMC2967023
6. Mathias, J.R., Dodd, M.E., Walters, K.B., **Rhodes, J.**, Kanki, J., Look, A.T., Huttenlocher, A. Live imaging of chronic inflammation caused by mutation of zebrafish Hai1. *J. Cell Sci*. **120**:3372-3383, 2007.
7. Walters, K.B., Dodd, M.E., Mathias, J.R., Gallagher, A.J., Bennin, D.A., **Rhodes, J.**, Kanki, J.P., Look, A.T., Grinblat, Y., Huttenlocher, A. Muscle degeneration and leukocyte infiltration caused by mutation of zebrafish fad24. *Dev. Dyn*. **238**:86-99, 2009. PMCID: PMC2843540.
8. Dodd, M.E., Hatzold, J., Mathias, J.R., Walters, K.B., Bennin, D.A., **Rhodes, J.,** Kanki, J.P., Look, A.T., Hammerschmidt, M., Huttenlocher, A. The ENTH domain protein Clint1 is required for epidermal homeostasis in zebrafish. *Development*. **136**:2591-600, 2009. PMCID: PMC2709065.
9. **Rhodes, J.**, Amsterdam, A., Sanda, T., Moreau, L.A., McKenna, K., Heinrichs, S., Ganem, N.J., Ho, K.W., Neuberg, D.S., Johnston, A., Ahn, Y., Kutok, J.L., Hromas, R., Wray, J., Lee, C., Murphy, C., Radke, I., Downing, J.R., Fleming, M.D., Macconaill, L.E., Amatruda, J.F., Gutierrez, A., Galinsky, I., Stone, R.M., Ross, E.A., Pellman, D.S., Kanki, J.P., Look, A.T. Emi1 maintains genomic integrity during Zebrafish embryogenesis and cooperates with p53 in tumor suppression. *Mol. Cell. Biol*. **29**:5911-5922, 2009. PMCID: PMC2772726.
10. Robinson, B.W., Germano, G., Song, Y., Abrams, J., Scott, M., Guariento, I., Tiso, N., Argenton, F., Basso, G., **Rhodes, J.**, Kanki, J.P., Look, A.T., Balice-Gordon, R.J., Felix, C.A. mll ortholog containing functional domains of human MLL is expressed throughout the zebrafish lifespan and in hematopoietic tissues. *Br. J. Haematol.* **152**:307-321, 2011. PMCID: PMC3158705.
11. Bolli, N., Payne, E.M., **Rhodes, J.**, Gjini, E., Johnston, A.B., Guo, F., Lee, J.S., Stewart, R.A. Kanki, J.P., Chen, A.T., Zhou, Y., Zon, L.I., Look, A.T. cpsf1 is required for definitive HSC survival in zebrafish. *Blood* **117**(15):3996-4007, 2011. PMCID: PMC3087528.
12. Payne, E.M., Bolli, N., **Rhodes, J.,** Abdel-Wahab, O.I., Levine, R., Hedvat, C.V., Stone, R., Khanna-Gupta, A., Sun, H., Kanki, J.P., Gazda, H.T., Beggs, A.H., Cotter, F.E., Look, A.T. Ddx18 is essential for cell-cycle progression in zebrafish hematopoietic cells and is mutated in human AML. *Blood.* **118**(4):903-915, 2011. PMCID: PMC3148170.
13. Robu, M.E., Zhang, Y., **Rhodes J.** Rereplication in emi1-deficient zebrafish embryos occurs through a cdh1-mediated pathway. *PLoS ONE*. **7**(10):e47658, 2012. PMCID: PMC3474755.
14. Zhang, Y., Duc, A., Rao, S., Sun. X.L., Bilbee, A.N., Rhodes, M.R., Li, Q., Kappes, D.J., *__Rhodes, J.__, *Wiest, D. Control of hematopoietic stem cell emergence by antagonistic functions of ribosomal protein paralogs. *Dev. Cell* **24**(4):411-425, 2013. PMCID: PMC3586312. *Authors contributed equally.