Novel method enabling the use of cryopreserved primary acute myeloid leukemia cells in functional drug screens

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Abstract

The ability to assess anti-leukemic drug activity on primary patient samples is a powerful tool in determining potential drug targets and selection of therapeutic agents with biologic and functional rationale. We previously established small-molecule inhibitor screens for use on freshly isolated leukemia cells for this purpose. Here we describe a method that produces functional small molecule inhibitor screening results using cryopreserved primary acute myeloid leukemia (AML) cells. This method was established to take advantage of bio-repositories containing archival material, such as those established by the Children's Oncology Group, and to enable validation of potential pathway dependencies uncovered by genomic analysis. Various conditions used to thaw and culture cryopreserved specimens were assessed for effect on viability, differentiation, and the ability to recapitulate sensitivity results obtained on fresh samples. The most reproducible results were obtained by quick-thawing and culturing samples in cytokine rich media prior to performing drug screens. Our data suggests cytokine-enriched media aids in maintaining the viability and numbers required to perform functional analysis on cryopreserved leukemia cells. This method can aid in producing informative data on therapeutic targeting and precision medicine efforts in leukemia by making use of bio-repositories and bio banks.

Keywords

Cryopreservation; Small molecule inhibitor assays; Acute myeloid leukemia (AML); Bio-repositories

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Authorship

M. D. designed and performed the research, interpreted data and wrote the manuscript. C.E.T. designed the research, wrote and edited the manuscript. A. A. created the FACS panel, provided guidance in performing the FACS experiment and edited the manuscript. K.T. provided preliminary data on cytokine media and edited the manuscript. S. M. and B. J. D. provided critical feedback and reviewed the manuscript.

Conflict of Interest Disclosure

The authors have no conflicts of interest to declare

Introduction

Cryopreservation is a commonly used technique for the transport and preservation of mononuclear cells (MNC) isolated from bone marrow and peripheral blood. Cryopreserved MNCs have many uses including: clinical testing, correlative studies for clinical trials, inclusion in bio-repositories, and post-transplant therapies. The Children's Oncology Group maintains 23,754 cryopreserved pediatric AML samples from 6,872 unique patients in a biobank established to provide insight into rare childhood cancers. Genetic data has been obtained on 3,393 of these tumors and our ability to identify inhibitor sensitivities has the ability to provide additional insight into novel mutation-drug associations in pediatric and adult AML. Initial attempts to thaw these samples were unsuccessful due to low viability and insufficient cell recovery. We sought to develop a method to optimize cell recovery from cryopreservation for use in small molecule inhibitor screens. Our overall goal was to facilitate functional validation of hypotheses generated from retrospective genomic analysis. Given the relative abundance of cryopreserved material our method could enable the expanded use of cryopreserved material from biorepositories and genetic studies.

Small molecule inhibitor panels can be used to uncover molecular targets essential for leukemia cell growth and have been successful in identifying effective therapies for patients¹⁻³. Furthermore, additional clinically relevant information can be gleaned by combining genetic data with functionally important targets identified by small molecule inhibitor screens⁴. Historically, we have used freshly isolated peripheral blood mononuclear cells (PBMCs) to perform inhibitor screens. However, the length of time currently required to obtain sequencing panel results is on the order of weeks, which is more time than is feasible to maintain primary cells in culture, thus necessitating the use of cryopreserved samples to functionally validate genomic findings.

Cryopreservation has the potential to induce phenotypic changes and can drastically decrease cell viability. Changes induced by cryopreservation have been explored for B-cells⁵⁻⁸, T-cells^{9,10} and other hematopoietic cell sub-populations^{11,12}. Therefore, evaluating the differences between freshly isolated and frozen cells is necessary to understand the potential effects that cryopreservation may have on downstream functional analyses.

We set out to overcome the low viability and poor cell recovery encountered with cryopreservation. Using media rich in hematopoietic growth factors, we tested the ability to support cell viability, maintain inhibitor sensitivity, and produce minimal changes in cell maturation markers. To empirically test each condition, cells were thawed and cultured in different mediums and assayed using a small molecule inhibitor panel. The results from inhibitor panel assays obtained from cryopreserved cells were compared to data obtained on freshly isolated cells. Concordance of functional results between fresh and frozen samples was used as a measure of reliability for each media. As distribution of cell maturation can be altered by cryopreservation^{11,12} and culturing in cytokine-enriched media, we assayed for alterations in specific cell surface maturation markers using fluorescence-activated cell sorting (FACS).

We report a method that maintains the highest viability and cell recovery, while minimizing changes in differentiation and functional screening results compared to fresh samples. This method supports the use of cryopreserved primary mononuclear cells (MNC) in small molecule inhibitor screens and could be extended to enable the use of cryopreserved cells in other downstream functional assays.

Material and Methods

Cell Preparations

Plasma was isolated from whole blood by centrifuging at 2,500 rpm for 10 minutes. The remaining blood was diluted with IMDM (Iscove's Modified Dulbecco's Medium): 1:1 for peripheral blood and 1:4 for bone marrow. MNC were separated using Ficoll-Paque PLUS (GE Healthcare Life Sciences, Pittsburgh, USA). Using a 15 mL Falcon Tube, 10 mL diluted blood or bone marrow was added dropwise to 4 mL Ficoll-paque and centrifuged at 1,200 rpm for 20 minutes with no break. MNC were isolated from the buffy coat for testing. Cell counts and viability were recorded using Guava ViaCount (Millipore, Billerica, USA). To create aliquots of frozen viable cells, ten million Ficoll separated MNC were re-suspended in 1 ml FBS, 10% DMSO, and gradually brought to -80°C , cooled at 1°C per min, using Mr. Frosty and isopropanol (Nalgene, New York, USA) according to manufacture specifications. After 24 hours, cells were transferred to liquid nitrogen for long-term storage.

Small Molecule Inhibitor Screens

Ficoll separated MNC were cultured in a 384-well plate small molecule inhibitor screen containing up to 20 tyrosine kinase inhibitors (TKIs) arrayed in 7-point dose gradients (Supplementary Table 1). In brief, 15,000 cells were plated per well in RPMI-1640 and 10% FBS with 2% L-Glutamine and 1% Penicillin/streptomycin (R10) with β -mercaptoethanol (BME) and incubated at 37°C , 5% CO_2 for 72 hours. A tetrazolium-based cell viability assay was performed using CellTiter 96 AQueous One Solution (MTS) (Promega, Madison, USA) according to manufacturer's protocol (Figure 1A). Absorbance was measured at 490nm using a BioTek Synergy 2 plate reader at 5 and 24 hours. Absorbance measured between 0.2–1.0 arbitrary units (AU) were considered within an acceptable range. Mitochondrial metabolic activity readouts over a given dose curve were used to calculate area under the curve (AUC) values. In some cases, drugs may alter mitochondrial function without affecting cell viability but we have historically assessed mitochondrial potential and used it as a surrogate for cell viability in our assay. AUC values were used as a measure of sensitivity to a given inhibitor.

Thawing and Culturing of Frozen Cells

Frozen cells were gradually warmed to 37°C until a small ice pellet remained. Thaw media (25% FBS in IMDM) warmed to 37°C was added drop-wise and the volume brought to 10 mL in a 15 mL conical tube. Cells were centrifuged at 1,000 rpm for 10 minutes and media was aspirated. Addition of thaw media, centrifugation and aspiration was repeated twice to remove residual DMSO. Cells were re-suspended in one of the following culture conditions: R10 (RPMI 10% FBS and 2% L- Glutamine and 1% Penicillin streptomycin), HS-5 condition media (HS-5 stromal cells were cultured in R10 for 72 hours, the media was

collected and filtered using a 0.45µm filter to remove residual cells, aliquots were frozen at -80 for future use), Stem Cell Media (SCM: IMDM containing 5% BIT9500, Penicillin streptomycin, 1% L-Glutamine, 10^{-4} M BME, 50pg/ml LIF, 1ng/ml G-CSF and IL-6, 0.2ng/ml GM-CSF, SCF, and MIP-1α), or Cytokine Culture Media (CCM: RPMI containing 25% FBS, 1% Penicillin streptomycin, 1% L-Glutamine, 10ng/ml IL-3, SCF, G-CSF, and GM-CSF; Figure 1B). Cells were cultured at a concentration of two million viable cells per mL for of 48 hours (37°C, 5% CO₂). After culturing, 5–7 million cells were centrifuged at 1,000 rpm for 10 minutes, re-suspended in R10, and plated onto inhibitor screen following the protocol outlined in the Small Molecule Inhibitor Screen section (above). Cells were incubated for three days at 37°C and viability was assessed using an MTS assay.

Area under the curve values were compared for inhibitors tested on both fresh and frozen cells for a given patient sample. Corresponding R² values were obtained using Prism (GraphPad CA, USA) to calculate linear regression.

Flow Cytometry

To examine the extent of cellular differentiation induced by cryopreservation and culturing, we assessed four conditions by FACS the relative levels of differentiation marker expression: 1) fresh cells cultured in R10, 2) fresh cells cultured in Cytokine Culture Media (CCM), 3) cryopreserved cells cultured in CCM, and 4) freshly thawed cryopreserved cells. All conditions were run simultaneously and compensation was set for each marker based on fresh cells cultured in R10. Cryopreserved samples were either frozen for 24 hours, thawed, and cultured in CCM for 48 hours or frozen for 72 hours, thawed, and immediately analyzed by FACS. Fresh cells were cultured for the 72 hours it took to adequately freeze, thaw, and culture the cryopreserved subset. Half a million viable cells were stained and gated using viability dye (BD Biosciences, VB510). Samples were analyzed for cell surface markers: CD45⁺, CD34⁺, CD14⁺, CD11b⁺, CD19⁺, CD3⁺, and CD13⁺CD33⁺ (BD Biosciences) to quantify the relative number of different cell types under each condition, including leukocytes, stem cells, monocytes, granulocytes, B-cells, T-cells and myeloid blasts respectively. Data were analyzed using FlowJo (Ashland, USA) and significance was measured using a one-way ANOVA Friedman test ($\alpha = 0.05$) and two-tailed nonparametric paired t-test in Prism (GraphPad CA, USA).

Results

Optimizing culture conditions for cryopreserved specimens

Culturing cryopreserved cells in cytokine-enriched media produced significantly better results than culturing in R10 alone prior to small molecule inhibitor screening (Figure 2A). Cells cultured in cytokine-enriched media for 48 hours post thaw resulted in the highest level of cell viability (Figure 2A; $p < 0.05$). Of the three cytokine mediums tested, Cytokine Culture Media (CCM) produced the highest concordance of drug responses when compared to results obtained on fresh samples (AUC; R² = 0.9251; Figure 2B), versus HS-5 conditioned media (R² = 0.7986; Figure 2C) and Stem Cell Media (SCM) (R² = 0.6978; Figure 2D). CCM proved to be the ideal media to progress with further testing due to high post-thaw viability and concordance with fresh inhibitor results. Despite the high viability of

HS5 cultured media, initial testing on inhibitor concordance was not as strong as CCM. Due to limited sample availability, we opted to discontinue testing with media other than CCM. Attempts to remove dead cells via Ficoll separation were unable to improve the concordance of the small molecule inhibitor assay results, likely due to continuous cell death over the 72 hour assay time period. Samples with poor viability consistently exhibited lower absorbance when measured, suggesting they did not survive the 72 hour incubation period required for the assay.

We thawed 36 cryopreserved primary AML samples from 17 individual patients to be cultured in CCM. From these 36 samples, 25 maintained cell viability greater than 70%, of these 17 maintained enough viable cells to plate on our inhibitor panel. Duplicate samples were tested to determine assay reproducibility between media types and sample status, fresh versus cryopreserved, a single representative sample run in all conditions of status and media type is shown in figure 3. We observed high concordance for duplicate plating of fresh samples assayed in R10 (Figure 3A; $R^2 = 0.9746$) and for duplicate plating of frozen samples thawed in CCM and then assayed in R10 (Figure 3B; $R^2 = 0.9794$). High correlation between fresh and frozen sample results was also observed (Figure 3C; $R^2 = 0.8606$). We tested a total of 17 samples cultured in CCM for 48 hours prior to plating in R10 on inhibitor plates, compare these results to data obtained on freshly plated cells, and obtained an average R^2 value of 0.6802 across all samples (Table 1). The most effective inhibitors, those with AUC values below 500nM had higher concordance, $R^2=0.7952$ (Table 1).

For each of the 17 cryopreserved samples tested on the inhibitor panel, we annotated leukemia subtype, sample origin (peripheral blood or bone marrow), number of days frozen, and percent blasts at time of draw. None one factor significantly correlated with the concordance of results between fresh and frozen samples (Table 1). We observed that plating cells immediately in R10 resulted in low concordant results between fresh and frozen cells, and rarely maintained viability greater than 70% after 48 hours.

For the 17 samples we tested, cryopreserved cells revived in CCM had an average AUC difference of 259.77 (AU) across all the drugs common between the fresh and frozen samples. Looking at inhibitors under 200 AUC from the fresh samples, the average difference between fresh and frozen inhibitors is 45.92 AU. “We observed no significant differences ($p<0.01$) when comparing the inhibitor AUC results for the fresh and frozen samples using a paired t-test Prism (GraphPad CA,USA) for 18 of 19 inhibitors. Significance was found between fresh and cryopreserved samples for the EGFR inhibitors Gefitinib ($n=17$, $p=0.0082$) and Lapatinib ($n=18$, $p=0.0475$), and a CSF1R inhibitor ($n=13$, $p=0.0074$) (Figure 4).

We examined the effects of cytokine media on inhibitor results by comparing fresh and cryopreserved cells from the same sample cultured in R10 and CCM (Figure 5). Cryopreserved cells cultured in CCM had the highest concordance with fresh cells cultured in R10 ($R^2 = 0.7195$; Figure 5A). Fresh cells and cryopreserved cells cultured in R10 and CCM (Figure 5B; $R^2=0.5266$ and 5C; $R^2=0.1503$ respectively) and thawed and fresh cells cultured in R10 alone (Figure 5D; $R^2=0.1928$) failed to produce higher levels of

concordance. Thawed cells cultured in R10 and run on an inhibitor panel did not survive the 72 hour culture period and resulted in average absorbance plate readings under 0.2 AU resulting in low concordance (Figure 5C, D).

We calculated the average AUC of all inhibitors tested on fresh and cryopreserved, CCM thawed, cells and performed a 2-way ANOVA and t-test using Prism test for significant differences. Of the 19 inhibitors tested, Gefitinib, Lapatinib, Array-382 were found to be significantly different between fresh and cryopreserved cells.

Cytokine Effects on Differentiation Status assessed by Flow Cytometry

To determine to what extent media and cryopreservation affected the differentiation status of the primary sample, we compared myeloid differentiation marker expression between freshly isolated cells and cryopreserved cells thawed in CCM and R10. Samples were stained with common leukocyte marker, CD45⁺, and myeloid differentiation markers: CD34⁺, CD14⁺, CD11b⁺, CD19⁺, CD3⁺, and CD13⁺CD33⁺. We observed no significant changes ($\alpha=0.05$) in marker expression across all conditions (Figure 6).

Discussion

Our experiments suggest that culturing cryopreserved cells in cytokine culture media (CCM) for at least 24 hours prior plating can support the cell viability required for analysis using small molecule inhibitor assays. Culturing in CCM generated results that most closely recapitulated those obtained using freshly isolated cells while culturing in non-cytokine enriched media proved ineffective. Based upon initial comparisons made by thawing cells in cytokine enriched media and R10, we concluded that R10 was insufficient to support the viability of cryopreserved cells and thereby limited the recovery of cells from cryopreservation. We opted to not select for blasts to maintain a diversity of cell types in our assay in order to observe the effects of non-blasts on inhibitor sensitivity.

Of the inhibitors that showed a significant difference between fresh and cryopreserved cells, Gefitinib and Lapatinib were ineffective in all samples tested. This is consistent with the low occurrence of mutations in the epidermal growth factor (EGFR) family in leukemia¹³. However, EGFR inhibitors have been found effective in patients who have had non-small cell lung cancer concurrent with AML^{14,15}. Leukemias that may respond to EGFR inhibitors like Gefitinib, additional work may be needed for analysis on cryopreserved cells. However, in the majority of leukemias that do not show sensitivity to this family of inhibitors, testing on cryopreserved cells may produce informative results. The CSF1R inhibitor was also found to be significantly different between fresh and cryopreserved.

Importantly, limited changes in cell populations were observed through FACS analysis when cells were cultured in cytokine media for 24 or 72 hours suggesting cryopreservation may have had little effect on the different cell sub-populations within the specimen. The limited duration of time in cytokine media appears to be sufficient in maintaining similar types and relative numbers of cells in the fresh and frozen samples. With no significant change in marker expression, we concluded that similar cell populations were being tested when running fresh and cryopreserved MNC.

Although we recognize that functional testing on fresh samples is the most ideal situation, in many instances this is not possible. Cryopreserved specimens provide a valuable resource that can be used to gain biological and functional information. Additionally, the wealth of samples maintained in bio-repositories can contribute to advancing precision medicine efforts in AML and other rare hematological malignancies. More specifically, functional screening performed on cryopreserved material has the ability to link genomic and functional targets and can aid in identifying patient-specific therapeutic strategies. While our work was focused on validation of results for small molecule inhibitor screens, we believe this method could also be useful for other assays that are able to capitalize on the wealth of data that could be obtained through the use of cryopreserved cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AML	Acute Myeloid Leukemia
AU	Arbitrary Units
AUC	Area under the curve
BME	β -mercaptoethanol
B-ALL	B lymphoblastic leukemia
BIT	Bovine serum albumin, Insulin and Transferrin
CCM	Cytokine Culture Media
CEM	Cytokine Enriched Media
COG	Children's Oncology Group
FACS	Fluorescence-activated cell sorting
FBS	Fetal Bovine Serum
IC50	Half maximal inhibitory concentration
IMDM	Iscove's Modified Dulbecco's Medium
MNC	Mononuclear cells
R10	Roswell Park Memorial Institute media with 10% Fetal Bovine Serum
RPMI	Roswell Park Memorial Institute

TKI tyrosine kinase inhibitors

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B.

Media Name	R10	SCM	CCM	HS-5	Thaw
Base Media	RPMI-1640	RPMI-1640	RPMI-1640	RPMI-1640	IMDM
Serum	10% FBS	5% BIT 9500	25% FBS	10% FBS	25% FBS
Cytokines		50pg/mL LIF 1ng/mL: G-CSF, IL-6 0.2ng/mL: GM-CSF, SCF, MIP 1α	10ng/mL: IL-3 SCF G-CSF GM-CSF	72hour collection of cytokines excreted from HS5 Stromal cells.	

Figure 1. Protocol to thaw and compare inhibitor results produced from viably frozen AML patient samples

A. Schema outlining the procedure for plating freshly isolated (FRESH) and cryopreserved (FROZEN) primary AML patient samples for testing in small molecule inhibitor screens. **B.** Table outlining the composition of the five different mediums utilized throughout these studies.

Figure 2. Effects of various culture conditions on viability and reproducibility of functional inhibitor screening data

A. Comparison of the percent viability of cryopreserved cells thawed and cultured in the four different media conditions. The number of replicate samples is indicated for each condition on the bar graph (**=p value<0.01). **B–D.** Comparison of average inhibitor area under the curve (AUC) values from functional screening data obtain on freshly isolated AML patient samples assayed in R10 media and cryopreserved cells thawed in **(B)** Cytokine Culture Media (CCM) media, **(C)** HS-5 conditioned media, and **(D)** Stem Cell Media (SCM) media. R^2 values were calculated by comparing data obtained from the fresh and the frozen cells for each media condition.

Figure 3. Comparison of inhibitor sensitivity AUCs obtained for freshly isolated and cryopreserved AML cells

We assessed the consistency of our method by comparing the AUCs of: **A.** a freshly isolated sample run in duplicate using the standard protocol for fresh cells ($R^2 = 0.9746$); **B.** a cryopreserved sample run in duplicate prepared with our proposed thaw protocol ($R^2 = 0.9794$); and **C.** comparison of a freshly isolated and cryopreserved counterpart from the same patient sample ($R^2 = 0.8606$).

Figure 4. Comparison of Area under the Curve between fresh and frozen samples by inhibitor
We compared area under the curve for each inhibitor with more than 3 points on between fresh and cryopreserved cells. Using a paired t-test, we found significant differences in 3 inhibitors, Gefitinib, Lapatinib, and a CSF1R inhibitor.

Figure 5. Effects of culturing primary cells in cytokine media on AUC values from inhibitor screens performed on primary cells

AML cells from a single patient were used to test the effects of media and cell status (fresh, cryopreserved) under four conditions 1) Fresh sample cultured in R10, 2) Fresh sample cultured in CCM, 3) cryopreserved sample cultured in R10, and 4) cryopreserved sample cultured in CCM. The following comparisons were made: **A.** Frozen AML patient samples thawed/cultured in CCM and plated in R10 compared to freshly isolated patient samples plated in R10 ($R^2 = 0.7195$). **B.** Freshly isolated samples plated in CCM compared to freshly isolated cells plated in R10 media ($R^2 = 0.5266$). **C.** Frozen patient samples cultured in CCM and plated in R10 compared to frozen cells plated in R10 ($R^2 = 0.1503$). **D.** Frozen cells thawed/cultured in R10 and plated in R10 compared to freshly isolated cells plated in R10 ($R^2 = 0.1928$).

Figure 6. Assessment of differentiation status by FACS analysis

To examine the relative change in cell differentiation status we performed FACS analysis on five samples, comparing fresh and frozen samples cultured in R10 and CCM. Seven cell surface markers (CD45+, CD34+, CD14+, CD11b+, CD19+, CD3+, and CD13+CD33+) were assessed to determine the relative changes in marker expression observed between fresh or cryopreserved cells grown in R10 or CCM. The error bars denote the standard deviation within surface marker. **A.** The number of CD45+ cell was determined for each sample/condition and plotted as a percentage of the total number of MNCs. **B.** The percent expression of the following markers: CD34+, CD14+, CD11b+, CD19+, CD3+, and CD13+CD33+ was determined and plotted as a percentage of the total CD45+ cell population for each sample/condition.

Table 1
Comparison of functional inhibitor screening results performed on freshly isolated and cryopreserved leukemia samples

Samples from each unique patient are given the same sample ID

#	Sample ID	Total Viable Cells	Viability	Specific Diagnosis	Type	Percent Blasts	Days Frozen	Average AUCR ²	Average AUCR ² (<500 AUC)
1	13-00092	1.92E+07	92.1	AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11	Peripheral Blood	86	574	0.7501	0.7200
2	13-00092	1.92E+07	92.1	AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11	Peripheral Blood	86	574	0.5643	0.7470
3	13-00195	4.21E+06	94.9	AML with mutated NPM1	Bone Marrow	90	505	0.6312	0.9829
4	13-00331	7.03E+05	79.4	AML with mutated NPM1	Peripheral Blood	92	679	0.3915	0.4599
5	13-00487	6.06E+06	93.6	AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11	Bone Marrow	90	119	0.9128	0.7710
6	13-00602	2.94E+07	90.5	AML with myelodysplasia-related changes	Peripheral Blood	85	72	0.4872	N/A
7	13-00603	3.05E+07	80.8		Peripheral Blood	85	72	0.8524	0.8932
8	13-00636	3.81E+06	87.6	AML with t(6;9)(p23;q34); DEK-NUP214	Bone Marrow	68	42	0.4647	0.8292
9	14-00044	1.06E+07	92.3	AML with mutated NPM1	Peripheral Blood	94	7	0.8811	0.9888
10	14-00045	9.03E+06	85.9	AML with myelodysplasia-related changes	Peripheral Blood	78	29	0.8525	0.8619
11	14-00045	6.56E+06	85.7	AML with myelodysplasia-related changes	Peripheral Blood	78	35	0.9551	0.9271
12	14-00331	7.90E+06	94.3	Acute myeloid leukaemia, NOS	Peripheral Blood	68	8	0.5879	0.9317
13	14-00331	7.95E+06	94.3	Acute myeloid leukaemia, NOS	Peripheral Blood	68	8	0.6112	0.9443
14	14-00356	1.63E+06	56.8	Acute monoblastic and monocytic leukaemia	Bone Marrow	88	26	0.5739	0.6052
15	14-00359	1.06E+07	64.8	AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11	Peripheral Blood	37	22	0.7673	0.6257
16	14-00423	9.36E+06	75.5	AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11	Peripheral Blood	61	140	0.7536	0.7518
17	15-00811	8.63E+06	81.0	AML with mutated NPM1	Peripheral Blood	13	1	0.5266	0.6831
	Averages	1.09E+07	84.8			74.5	171	0.6802	0.7952

Total Viable = total number of viable cells available prior to plating. **Viability 24 hours** = percent viable cells after thawing and culturing CCM for 24 hours. **Type** = the type of sample (peripheral blood or bone marrow) that was used to isolate cells for the assay. **Percent Blasts** = percentage of circulating blasts in the patient at the time of sample acquisition as assessed by Flow cytometry. **Days frozen** = number of days that the cryopreserved sample was stored in liquid nitrogen prior to thawing. **Area Under the Curve $R^2 = R^2$** values obtained from a comparison of the fresh and frozen sample inhibitor area under the curve (AUC) values for each given drug.