Final Project

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Project Outline

Acute myeloid leukemia (AML) is a clinically devastating cancer (<30% 5 year-survival rate) that has been extraordinarily difficult to treat due to its complex genetic subtypes. One shared hallmark of AML is the arrest of leukemic myeloblasts at an immature and self-renewing stage of development. Therapies that are able to overcome this arrest represent a powerful treatment strategy. Sykes et al developed a cellular model of HoxA9-enforced myeloid differentiation arrest to use in an unbiased phenotypic flow-cytometry based screen. In this system, 96-well and 384-well plates are treated with titrated small compounds in order to assess viability, changes in cellular phenotype (based on cell surface markers), and differentiation status (ie: are the cells able to overcome their differentiation arrest to take on a neutrophil phenotype versus a myeloblast progenitor phenotype). Compounds that allow for differentiation are considered "hits" in this system and should be further studied to identify details on mechanism of action and pharmacokinetics. Developing code to analyze the output (over 330,000 compounds will need to be tested at multiple doses) will be essential for quickly identifying "hits".

Import File

Import Excel file for analysis. The goal will be to set up a platform to analyze flow cytometry data from 96-well and 384-well plates for cell viability (determine whether compounds are overly toxic) and cell surface markers (determine changes in differentiation status) caused by treatment with DHODH inhibitor compounds.

Determine Viability Normalized to Control

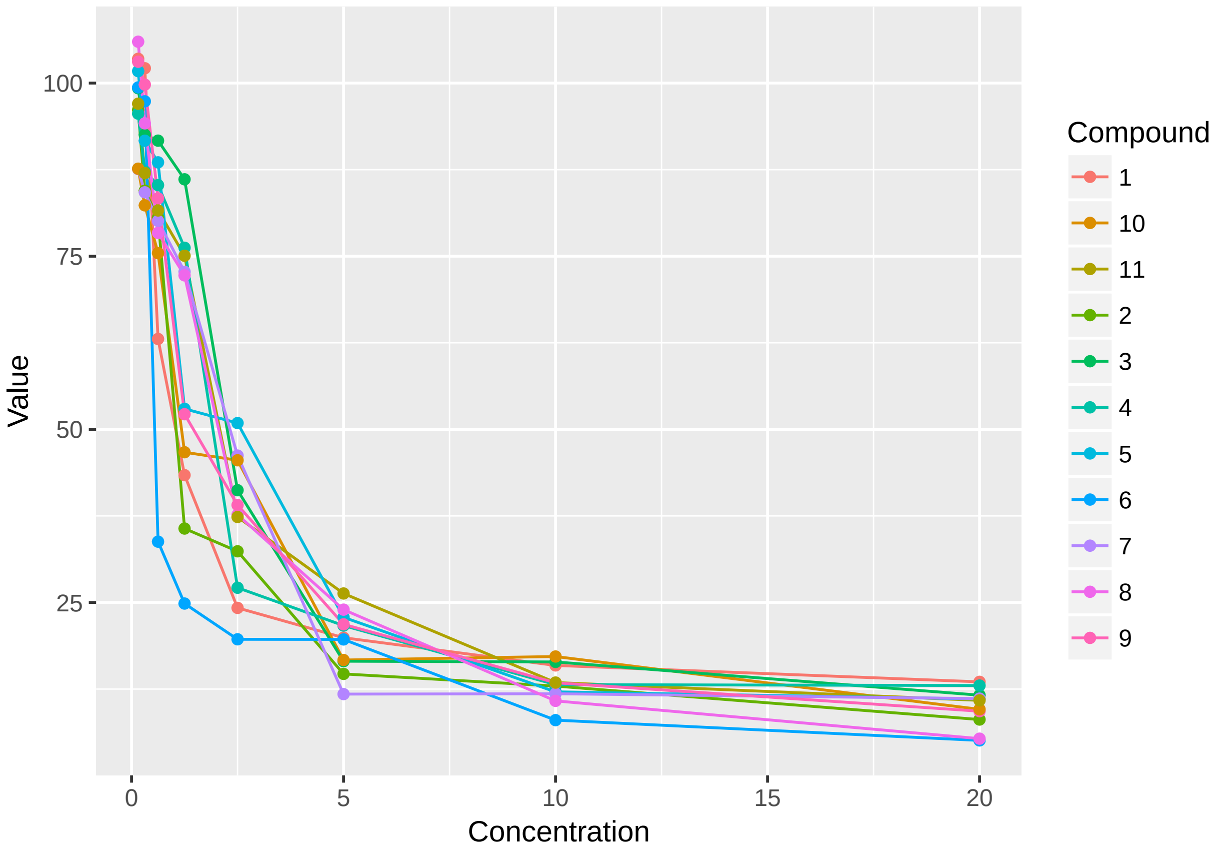
Calculate viability as a percentage normalized to an average of the DMSO wells.

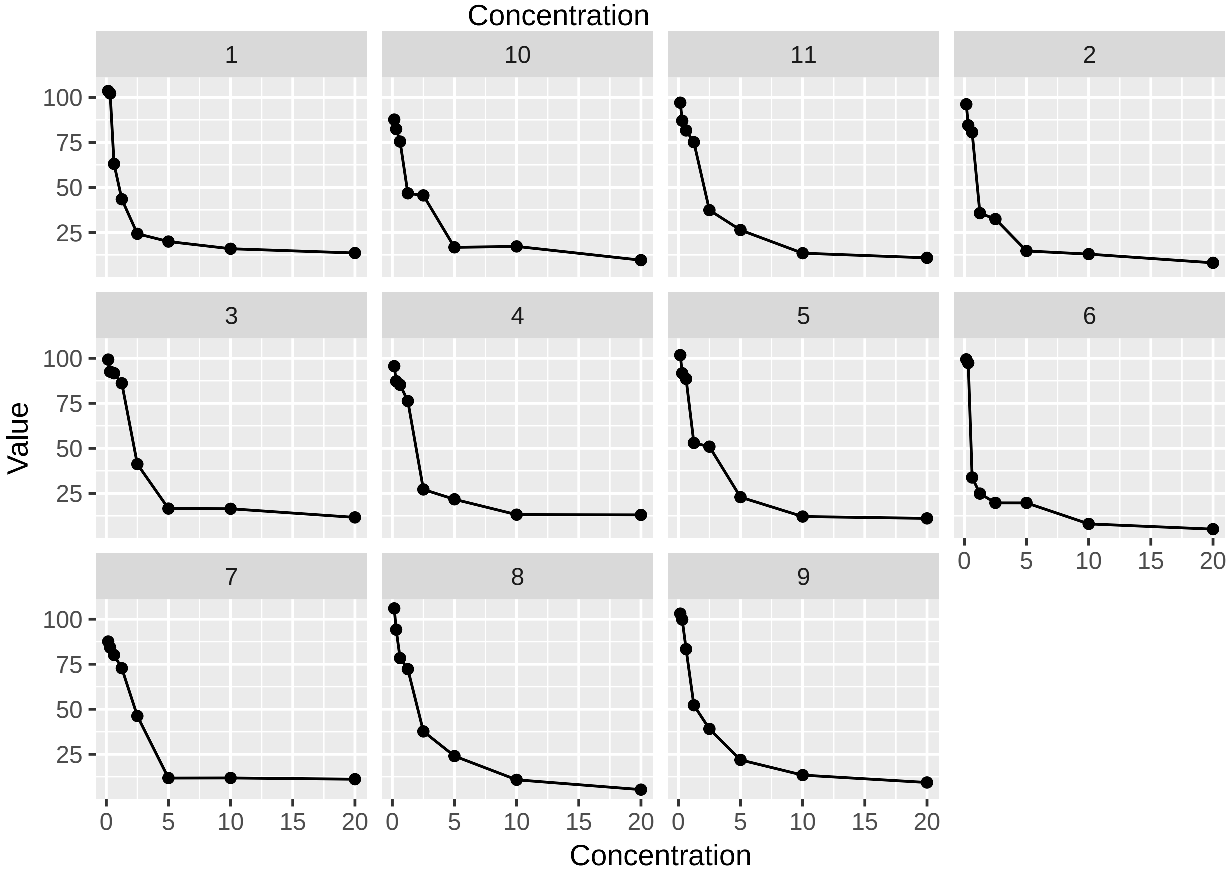
```
##   Compound.1 Compound.2 Compound.3 Compound.4 Compound.5 Compound.6
## 1      13.54       8.10      11.61      13.01      11.07       5.08
## 2      15.91      12.93      16.42      13.14      12.09       8.01
## 3      19.91      14.68      16.52      21.67      22.86      19.66
## 4      24.22      32.39      41.21      27.13      50.91      19.68
## 5      43.39      35.68      86.10      76.22      52.96      24.84
## 6      63.05      80.58      91.69      85.26      88.55      33.79
## 7     102.14      84.46      92.54      87.24      91.67      97.37
## 8     103.51      96.14      99.26      95.59     101.72      99.37
##   Compound.7 Compound.8 Compound.9 Compound.10 Compound.11   DMSO
## 1      11.12       5.34       9.30        9.57       10.87  92.00
## 2      11.82      10.79      13.40       17.20       13.44 103.55
## 3      11.77      23.97      21.84       16.69       26.30  91.14
## 4      46.21      37.67      39.07       45.53       37.34  99.37
## 5      72.76      72.23      52.18       46.69       75.07  93.57
## 6      80.11      78.37      83.35       75.44       81.61 104.88
## 7      84.23      94.18      99.78       82.35       87.01 110.41
## 8      87.59     105.97     103.08       87.64       97.02 105.09
```

Viability Curves

```
## ------------------------------------------------------------------------
## data.table + dplyr code now lives in dtplyr.
## Please library(dtplyr)!
## ------------------------------------------------------------------------
##
## Attaching package: 'data.table'
## The following objects are masked from 'package:dplyr':
##
##     between, first, last
## The following object is masked from 'package:purrr':
##
##     transpose
##     Concentration      DMSO    variable      value Compound
##  1:      20.00000  91.99632  Compound.1  13.540708        1
##  2:      10.00000 103.54761  Compound.1  15.909614        1
##  3:       5.00000  91.13960  Compound.1  19.911454        1
##  4:       2.50000  99.37328  Compound.1  24.218031        1
##  5:       1.25000  93.56601  Compound.1  43.387764        1
##  6:       0.62500 104.87580  Compound.1  63.051978        1
##  7:       0.31250 110.41283  Compound.1 102.138914        1
##  8:       0.15625 105.08855  Compound.1 103.507360        1
##  9:      20.00000  91.99632  Compound.2   8.095676        2
## 10:      10.00000 103.54761  Compound.2  12.925483        2
## 11:       5.00000  91.13960  Compound.2  14.684913        2
## 12:       2.50000  99.37328  Compound.2  32.388454        2
## 13:       1.25000  93.56601  Compound.2  35.677323        2
## 14:       0.62500 104.87580  Compound.2  80.577277        2
## 15:       0.31250 110.41283  Compound.2  84.458372        2
## 16:       0.15625 105.08855  Compound.2  96.136155        2
## 17:      20.00000  91.99632  Compound.3  11.614535        3
## 18:      10.00000 103.54761  Compound.3  16.415593        3
## 19:       5.00000  91.13960  Compound.3  16.519089        3
## 20:       2.50000  99.37328  Compound.3  41.208602        3
## 21:       1.25000  93.56601  Compound.3  86.102806        3
## 22:       0.62500 104.87580  Compound.3  91.685833        3
## 23:       0.31250 110.41283  Compound.3  92.542548        3
## 24:       0.15625 105.08855  Compound.3  99.258280        3
## 25:      20.00000  91.99632  Compound.4  13.005980        4
## 26:      10.00000 103.54761  Compound.4  13.143974        4
## 27:       5.00000  91.13960  Compound.4  21.665133        4
## 28:       2.50000  99.37328  Compound.4  27.127415        4
## 29:       1.25000  93.56601  Compound.4  76.218951        4
## 30:       0.62500 104.87580  Compound.4  85.263339        4
## 31:       0.31250 110.41283  Compound.4  87.235511        4
## 32:       0.15625 105.08855  Compound.4  95.589926        4
## 33:      20.00000  91.99632  Compound.5  11.068307        5
## 34:      10.00000 103.54761  Compound.5  12.091766        5
## 35:       5.00000  91.13960  Compound.5  22.855336        5
## 36:       2.50000  99.37328  Compound.5  50.908464        5
```

```
## 37:       1.25000  93.56601  Compound.5  52.961132        5
## 38:       0.62500 104.87580  Compound.5  88.552208        5
## 39:       0.31250 110.41283  Compound.5  91.674333        5
## 40:       0.15625 105.08855  Compound.5 101.724931        5
## 41:      20.00000  91.99632  Compound.6   5.082797        6
## 42:      10.00000 103.54761  Compound.6   8.009430        6
## 43:       5.00000  91.13960  Compound.6  19.664213        6
## 44:       2.50000  99.37328  Compound.6  19.681463        6
## 45:       1.25000  93.56601  Compound.6  24.844756        6
## 46:       0.62500 104.87580  Compound.6  33.785649        6
## 47:       0.31250 110.41283  Compound.6  97.366605        6
## 48:       0.15625 105.08855  Compound.6  99.373275        6
## 49:      20.00000  91.99632  Compound.7  11.120055        7
## 50:      10.00000 103.54761  Compound.7  11.821527        7
## 51:       5.00000  91.13960  Compound.7  11.769779        7
## 52:       2.50000  99.37328  Compound.7  46.210902        7
## 53:       1.25000  93.56601  Compound.7  72.757590        7
## 54:       0.62500 104.87580  Compound.7  80.105796        7
## 55:       0.31250 110.41283  Compound.7  84.228381        7
## 56:       0.15625 105.08855  Compound.7  87.591996        7
## 57:      20.00000  91.99632  Compound.8   5.335787        8
## 58:      10.00000 103.54761  Compound.8  10.792318        8
## 59:       5.00000  91.13960  Compound.8  23.965041        8
## 60:       2.50000  99.37328  Compound.8  37.666743        8
## 61:       1.25000  93.56601  Compound.8  72.234361        8
## 62:       0.62500 104.87580  Compound.8  78.369365        8
## 63:       0.31250 110.41283  Compound.8  94.181233        8
## 64:       0.15625 105.08855  Compound.8 105.974011        8
## 65:      20.00000  91.99632  Compound.9   9.303128        9
## 66:      10.00000 103.54761  Compound.9  13.402714        9
## 67:       5.00000  91.13960  Compound.9  21.843376        9
## 68:       2.50000  99.37328  Compound.9  39.069687        9
## 69:       1.25000  93.56601  Compound.9  52.184913        9
## 70:       0.62500 104.87580  Compound.9  83.348666        9
## 71:       0.31250 110.41283  Compound.9  99.775759        9
## 72:       0.15625 105.08855  Compound.9 103.081877        9
## 73:      20.00000  91.99632 Compound.10   9.567617       10
## 74:      10.00000 103.54761 Compound.10  17.197562       10
## 75:       5.00000  91.13960 Compound.10  16.691582       10
## 76:       2.50000  99.37328 Compound.10  45.532429       10
## 77:       1.25000  93.56601 Compound.10  46.693882       10
## 78:       0.62500 104.87580 Compound.10  75.442732       10
## 79:       0.31250 110.41283 Compound.10  82.348206       10
## 80:       0.15625 105.08855 Compound.10  87.637994       10
## 81:      20.00000  91.99632 Compound.11  10.867065       11
## 82:      10.00000 103.54761 Compound.11  13.437213       11
## 83:       5.00000  91.13960 Compound.11  26.299448       11
## 84:       2.50000  99.37328 Compound.11  37.344756       11
## 85:       1.25000  93.56601 Compound.11  75.068997       11
## 86:       0.62500 104.87580 Compound.11  81.606486       11
## 87:       0.31250 110.41283 Compound.11  87.005520       11
## 88:       0.15625 105.08855 Compound.11  97.021619       11
##     Concentration      DMSO    variable      value Compound
```


Compound
1
10
11
2
3
4
5
6
7
8
9
Value
Concentration


1
10
11
2
3
4
5
6
7
8
9
Value
Concentration

Calculate IC50

Calculate the concentration at which 50% of the cells are dead. This value can be used in further validation studies.

1. Take concentration value from each graph coresponding to 50% on y-axis
2. Convert Concentration in uM to logM

Calculate Differentiation

Determine which compounds cause differentiation of myeloblasts by calculating the percent of cells double-positive for Cd11b and GR-1, two cell surface markers of mature myeloid cells.