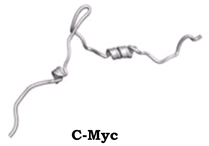

C-Myc

Lost in Transcription

Finding Potential Small Molecule Inhibitors of Transcription Factor C-Myc

Rachel, Dina Wassaf[1], Dominick Casalena[1], Angela Koehler[1, 2], Stuart Schreiber[1,2]

The Broad Institute[1], Harvard University[2], Cambridge, MA


Myc/Max

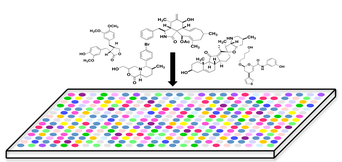
Introduction

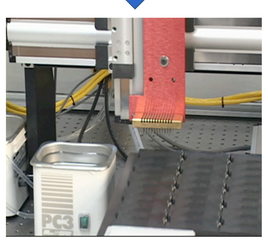
• **What is c-Myc?** C-Myc is a transcription factor; it binds to DNA to control the transcription of DNA into mRNA. C-Myc regulates many genes, including those involved in cell proliferation.
• **Why screen c-Myc?** Over-expression of c-Myc is found in cancer cells. Inhibiting c-Myc when it is up regulated could control cancerous behavior.

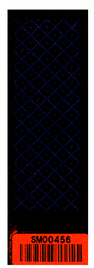
• **What is the goal?** To find a small molecule inhibitor of c-Myc. Because the c-Myc protein is Intrinsically Disordered, it has no stable structure and is unable to function when it is not bound to another protein. C-Myc typically binds with another transcription factor, Max. If a small molecule is found to bind to c-Myc, this molecule could prevent c-Myc's coupling with Max and inhibit the transcription of genes relating to cell proliferation.

• **What will be done?** C-Myc will be screened against over 20,000 unique small molecules to search for binders using a Small Molecule Microarrays (SMM).
• **Why use SMM?**
  • **It's very high throughput:** 20,000 compounds can be tested in a few hours. Because the structure of c-Myc is so complex, screening the protein against a large library of compounds is necessary to find binders.
  • **It's cheap:** SMM use minimal amounts of reagents.
  • **The compounds are diverse:** These SMM slides are printed with members of multiple libraries of compounds. The compounds are structurally diverse and many have not previously been tested in other assays.
  • **It's sensitive:** SMM are capable of finding low affinity binders that may be missed in other types of screening assays.

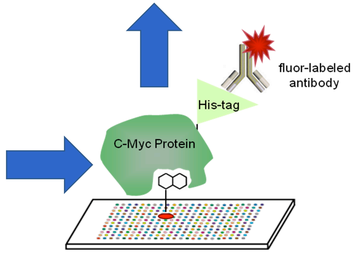
Methods


Stock Solutions


Slide Printing with a Small Molecule Microarrayer



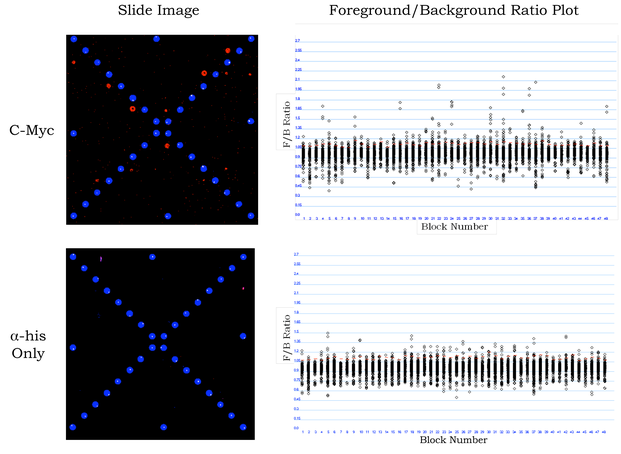
Slide Reading and Data Analysis



Protein and Antibody Incubations

Results and Conclusions

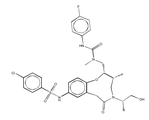
The C-Myc screen had a 1.3% hit rate



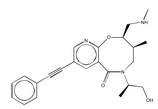
Commonly Used Terms
• **α-his:** The antibody that binds with the his tag on the c-Myc protein
• **F/B Ratio:** A ratio of the signal of a spot on slide (foreground) over the signal of the area around the spot (background)
• **Average Signal:** The mean signal of all four hits of a given compound.
• **Variance:** Describes the difference between the signal of one hit from the average signal of the compound
• **%CV:** A measure of variance expressed as a percent difference from the mean signal

Hits with Highest Signal

Average Signal: 3.58
%CV: 19.09


BRD-K59626414-001-01-5

Average Signal: 2.85
%CV: 3.66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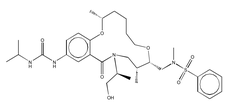
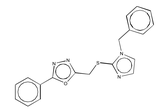
Average Signal: 2.59
%CV: 18.46

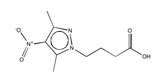

BRD-K27638954-001-01-5

Hits with Lowest Variance

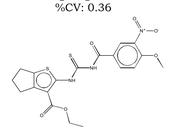
Average Signal: 1.18
%CV: 0.25


BRD-K80731058-001-01-7

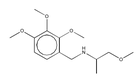
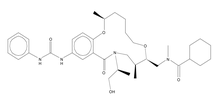
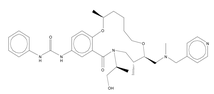
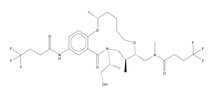
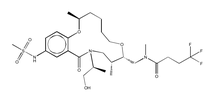
Average Signal: 1.15
%CV: 0.35


BRD-K31851742-001-01-5

Average Signal: 1.25
%CV: 0.36


BRD-K63245238-001-01-2

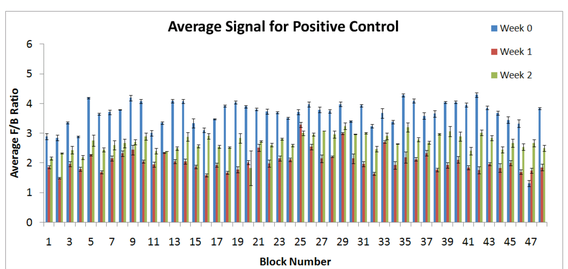
Three Largest Structural Clusters

	Highest Signal	Lowest Variance
Cluster 1 **Members: 17** **Average Signal: 1.22**	Average Signal: 1.53 %CV: 3.09  BRD-A43087975-001-01-2	Average Signal: 1.20 %CV: 0.76  BRD-A47587331-001-01-6
Cluster 2 **Members: 13** **Average Signal: 1.26**	Average Signal: 2.22 %CV: 26.58  BRD-K14621763-001-01-7	Average Signal: 1.17 %CV: 1.60  BRD-K25070860-001-01-6
Cluster 3 **Members: 10** **Average Signal: 1.26**	Average Signal: 2.18 %CV: 11.24  BRD-K53511806-001-01-1	Average Signal: 1.17 %CV: 1.49  BRD-K47825575-001-01-3

Assay Development

Chip Stability

SMM technology is relatively new. Sometimes there will be variability between screens and the reasons for it are unclear. A 12-week study has been launched to test the effects of chip storage. A large batch of chips was made and stored. Every other week, a few chips from this batch are screened against FKBP12, a protein with a known positive control (rapamycin) on SMM.



The Data Show:
• There is a drop in signal from Week 0 to Week 1.

• Signal goes up for Week 2, but not back to Week 0 levels.

Speculation:
• Signal goes down after Week 1 and plateaus.

• Discrepancy between Weeks 1 and 2 is normal variation.

These patterns might become clearer after more data have been collected.

Future Directions

• **Affinity Testing:** Surface Plasmon Resonance could be used to test the affinity of the hits called from the SMM screen. This could determine which compounds might be most useful and will help to remove false positives.
• **DNA Binding Assays:** A DNA binding assay could be done to test if a small molecule prevents Myc/Max from binding to DNA.
• **Functional Assays:** A cell-based assay should be done to determine if the small molecule hits from the SMM screen actually inhibit the c-Myc protein in a biologically relevant environment. Also, performing additional assays on *other* proteins that exist *in vivo* with c-Myc could show which compounds are non-specific binders.
• **Screening Max and Myc/Max:** Attacking this problem from different angles could be useful. SMM screens of the Max protein could be done to find small molecules that inhibit Max's binding to Myc. SMM screens of the Myc/Max heterodimer could be conducted to see if there are small molecules that will prevent the two protein partners from binding to DNA and thus halt transcription.
• **Developing New Cancer Treatments:** Eventually, some of these small molecules could be used as drugs for cancer. More tests – such as the screens listed above, studies on mice, and clinical trials – are needed to get to this point.

Acknowledgements

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