

Analyzing the Variation in Melanoma Cell Phenotype to Observe Homeostatic Changes to Cell State

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Introduction

- Wrangle and clean the dataset provided which contains the different protein and transcription levels at varying dose, time, and drug treatment
- Explore the various relationships between the MiTFg, SOX10, NGFR, AXL proteins and the 22 transcription factors
- Interpret the results and relate them to the 4 different melanoma cell phenotypes
- Form a general hypothesis about how these different factors and variables can cause different homeostatic changes that result in harmful or beneficial phenotypes

Objectives

- ① Observe how protein levels in an arbitrary experimental condition change over time
- ② Determine the relationship between different proteins at varying experimental conditions at times
- ③ Create a model predicting cellular phenotypical outcomes or values/states from transcription factors
- ④ Decipher the results and conclude any patterns or commonalities between the analyses

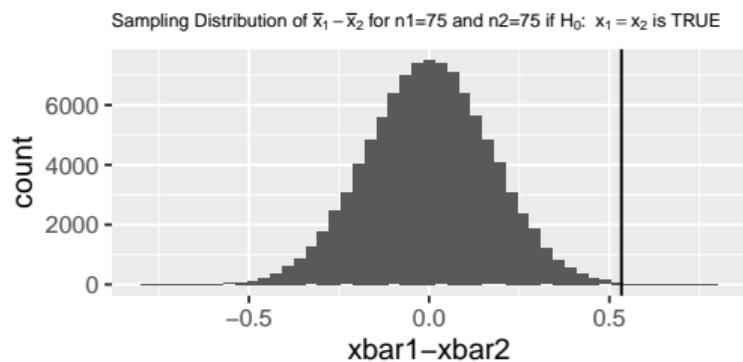
Statistical Methods

Throughout the analysis we will use various techniques to come to conclusions:

- ① Hypothesis Testing
- ② Correlation Estimation
- ③ Classification Modelling

MiTFg with Ven: 0.316 uM dose, 0.5h vs. 120h

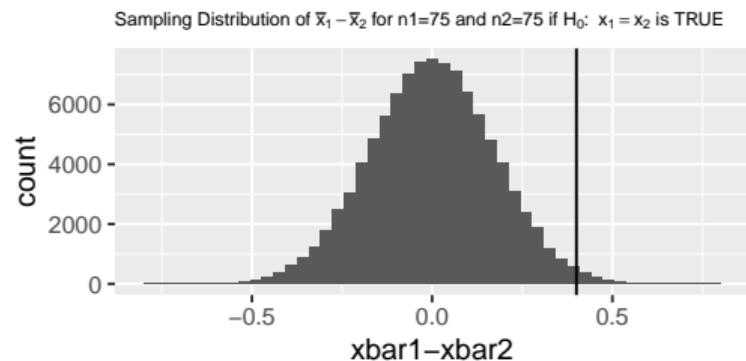
- H_0 : There is no change in the level of MiTFg protein between times when Vem is administered
- H_a : There is a change in the level of MiTFg protein between times when Vem is administered
- At the alpha significance level of 0.01, we have enough evidence to reject the null hypothesis and conclude there is a difference in MiTFg level at different times



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## [1] 0.00157
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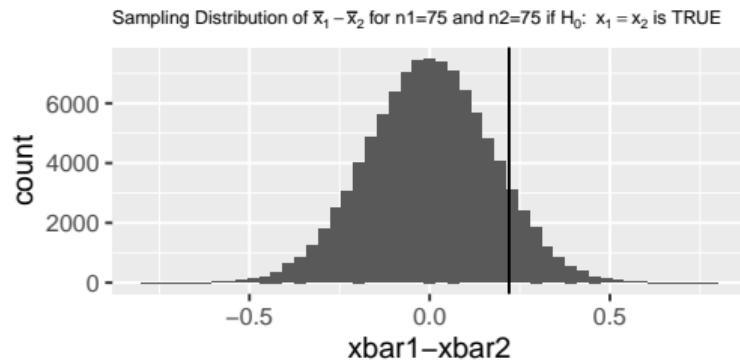
MiTFg with Vem+Tram: 0.316 μ M dose, 0.5h vs. 120h

- H_0 : There is no change in the level of MiTFg protein between times when Vem and Tram is administered
- H_a : There is a change in the level of MiTFg protein between times when Vem and Tram is administered
- At the alpha significance level of 0.01, we don't have enough evidence to reject the null hypothesis



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## [1] 0.01964
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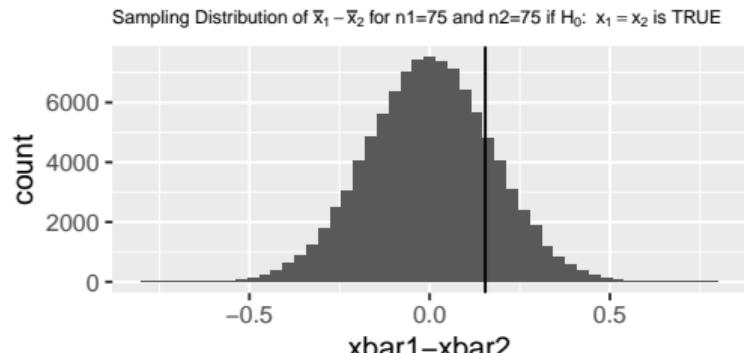
SOX10 with Vem: 0.316uM dose, 0.5h vs. 120h



- H_0 : There is no change in the level of SOX10 protein between times when Vem is administered
- H_a : There is a change in the level of SOX10 protein between times when Vem is administered
- At the alpha significance level of 0.01, we don't have enough evidence to reject the null hypothesis

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## [1] 0.20047
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SOX10 with Vem+Tram: 0.316uM dose, 0.5h vs. 120h

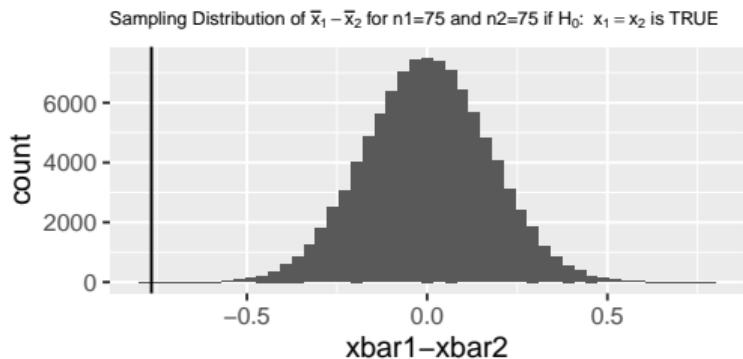


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## [1] 0.37116
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- H_0 : There is no change in the level of SOX10 protein between times when Vem and Tram is administered
- H_a : There is a change in the level of SOX10 protein between times when Vem and Tram is administered
- Similarly, at the alpha significance level of 0.01, we don't have enough evidence to reject the null hypothesis

NGFR with Vem: 0.316uM dose, 0.5h vs. 120h

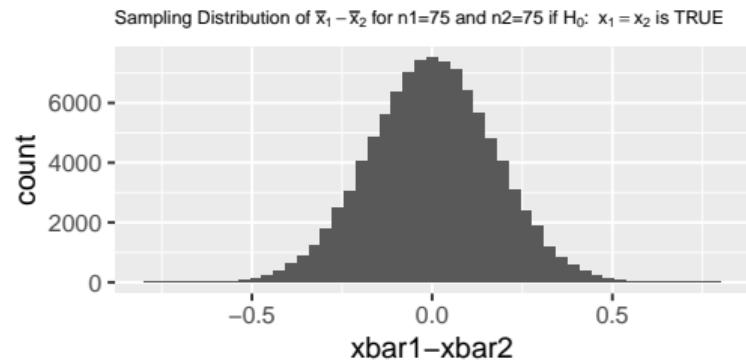
- H_0 : There is no change in the level of NGFR protein between times when Vem is administered
- H_a : There is a change in the level of NGFR protein between times when Vem is administered
- At the alpha significance level of 0.01, we have overwhelming evidence to reject the null hypothesis and conclude that there is a change in NGFR level between times when Vem is administered



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## [1] 1e-05
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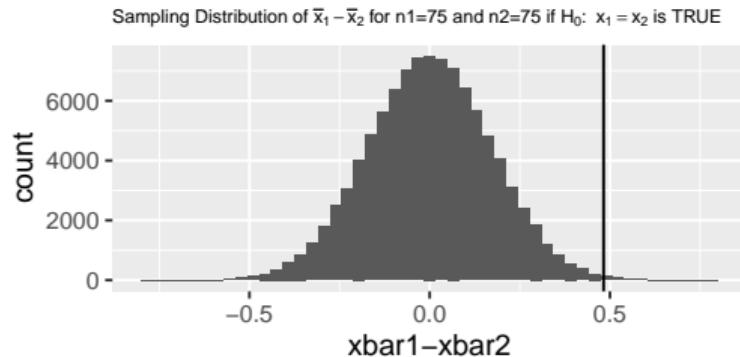
NGFR with Vem+Tram: 0.316 μ M dose, 0.5h vs. 120h

- H_0 : There is no change in the level of NGFR protein between times when Vem and Tram is administered
- H_a : There is a change in the level of NGFR protein between times when Vem and Tram is administered
- Similarly, at the alpha significance level of 0.01, we have overwhelming evidence to reject the null hypothesis and conclude that there is a change in NGFR level between times when Vem and Tram is administered



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## [1] 0
```

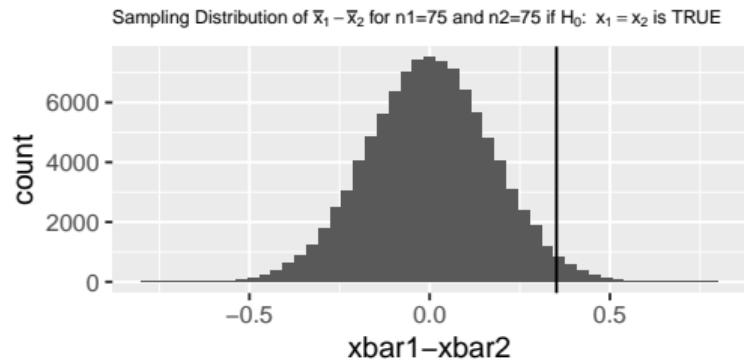
AXL with Vem: 0.316uM dose, 0.5h vs. 120h



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## [1] 0.00469
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- H_0 : There is no change in the level of AXL protein between times when Vem is administered
- H_a : There is a change in the level of AXL protein between times when Vem is administered
- At the alpha significance level of 0.01, we have evidence to reject the null hypothesis and conclude that there is a change in AXL level between times when Vem is administered

AXL with Vem+Tram: 0.316uM dose, 0.5h vs. 120h

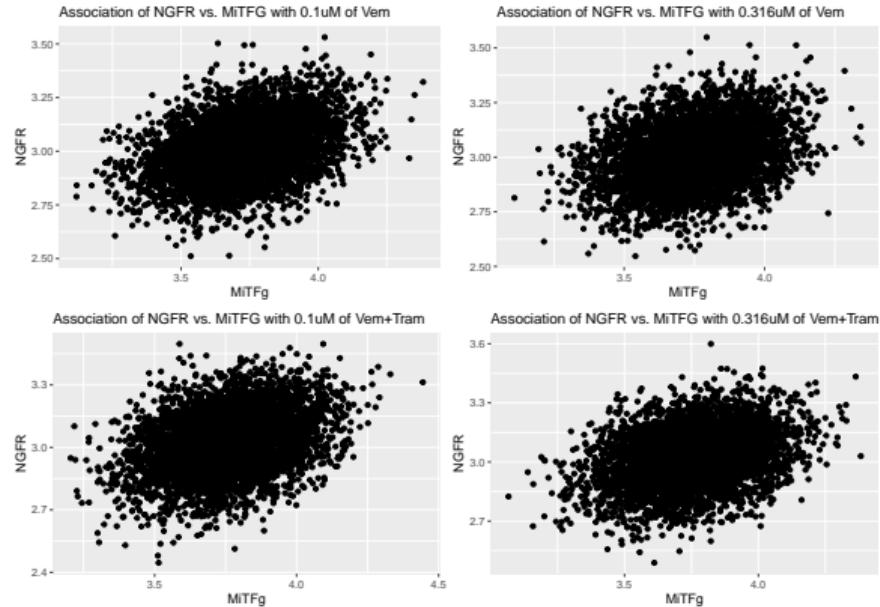


- H_0 : There is no change in the level of AXL protein between times when Vem is administered
- H_a : There is a change in the level of AXL protein between times when Vem is administered
- At the alpha significance level of 0.01, we don't have evidence to reject the null hypothesis

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## [1] 0.04093
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Correlation between MiTFg and NGFR

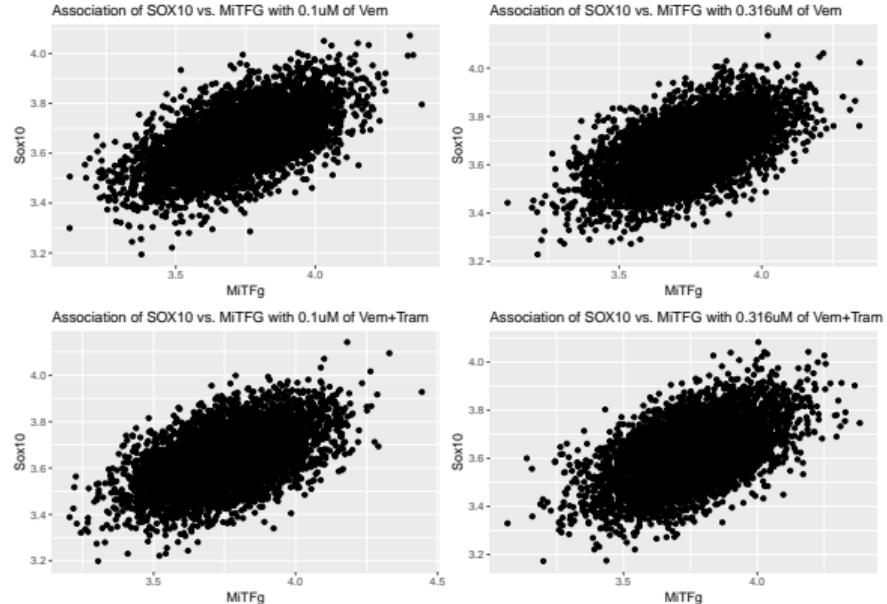
- We measure varying dosage and drug treatment at a set time
- The correlation between MiTFg and NGFR is relatively weak
- As dosage is increased, protein levels of NGFR slightly decrease in relation to MiTFg, however, when Vem+Tram is used, NGFR increases



	0.1 uM	0.316uM
Vem	0.3620898	0.3384028
Vem + Tram	0.2984683	0.3127059

Correlation between MiTFg and SOX10

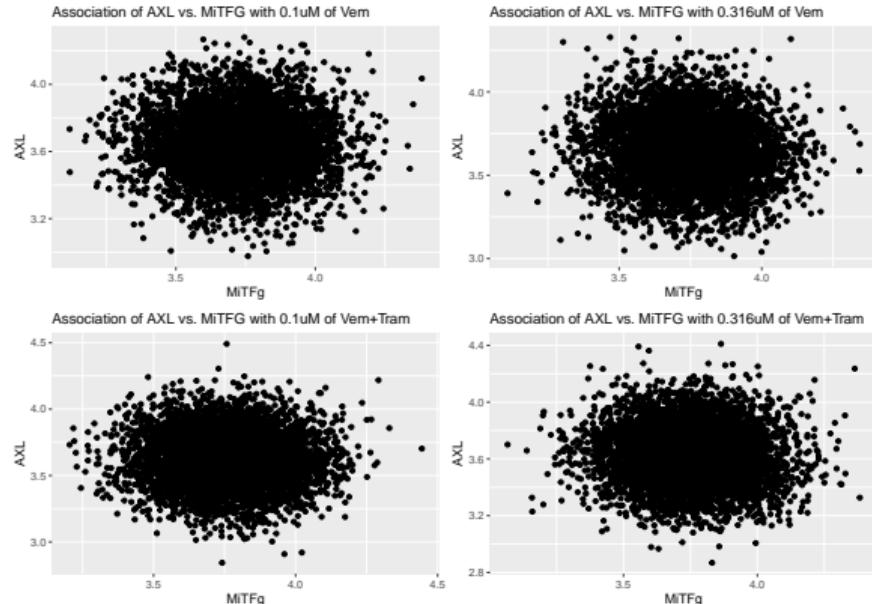
- The relationship between MiTFg and SOX10 is relatively stronger
- Correlation seems to become weaker for Vem but stays the same for Vem and Tram
- The level of SOX10 decreases as dosage of Vem is increased in relation to MiTFg. When Vem+Tram is used, protein level stays the same



	0.1 uM	0.316uM
Vem	0.5793084	0.5259164
Vem + Tram	0.5561515	0.5584327

Correlation between MiTFg and AXL

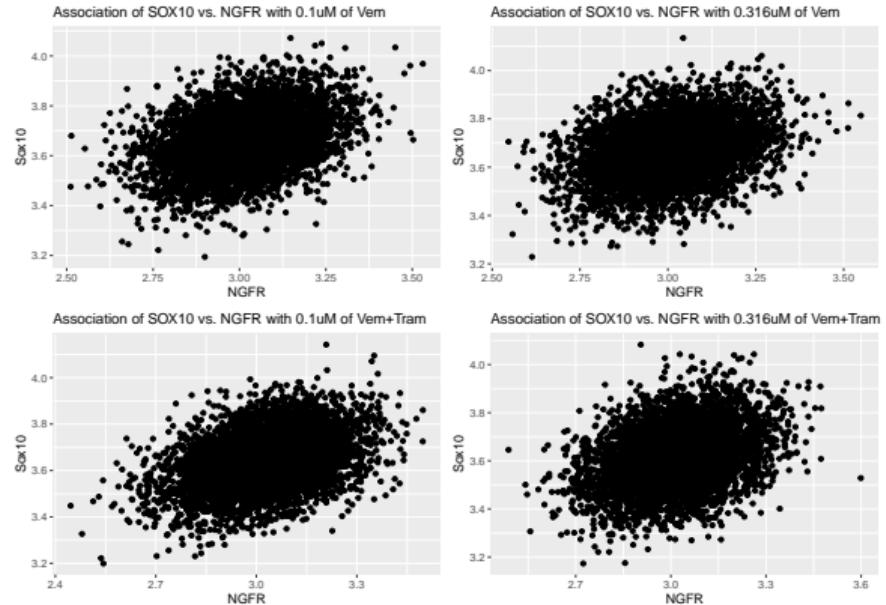
- As shown by the table, there seems to be no correlation between MiTFg and AXL
- This means that they would not be good predictors of each other
- Protein level of AXL roughly stays the same in relation to MiTFg when dosage is increased for both drug treatments



	0.1 uM	0.316uM
Vem	-0.0340941	-0.0329447
Vem + Tram	-0.0765529	-0.0545494

Correlation between NGFR and SOX10

- The relationship between NGFR and SOX10 is relatively weak
- The r coefficient increases with dose for both drug treatments
- As dosage of both Vem and Vem+Tram is increased, the level of SOX10 increases in relation to NGFR

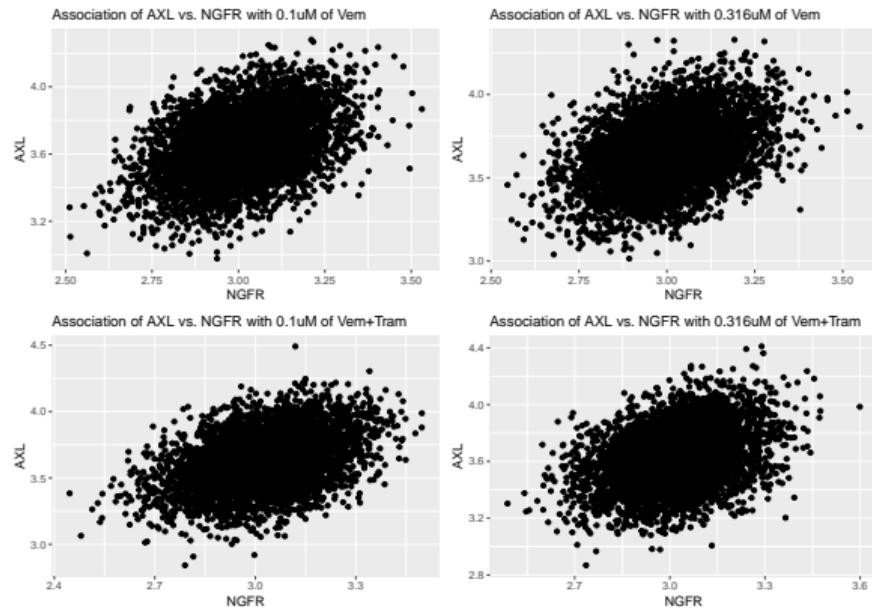


	0.1 uM	0.316uM
Vem	0.3595573	0.3866196
Vem + Tram	0.3121921	0.3316243

Correlation between NGFR and AXL

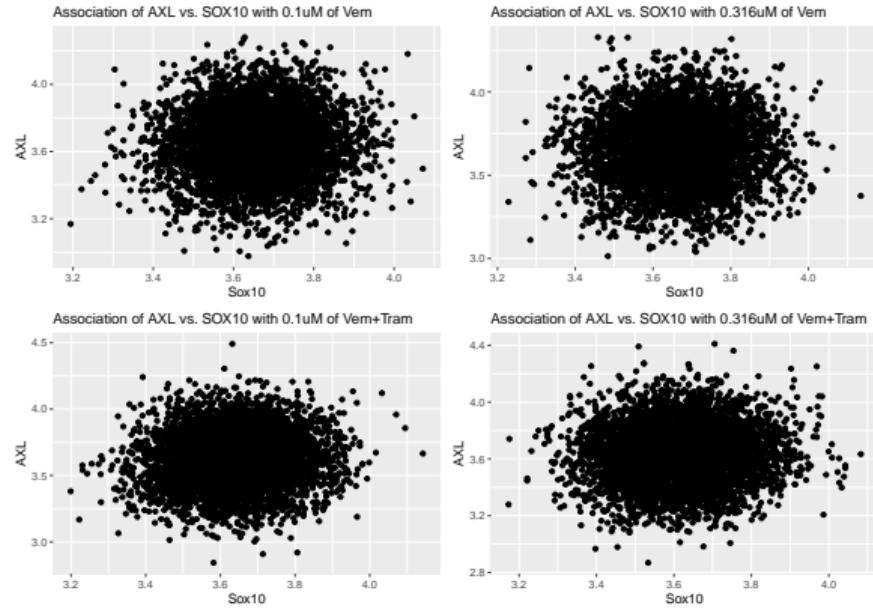
- The relationship between NGFR and AXL is relatively weak
- The r coefficient decreases with dose for both drug treatments
- The level of AXL decreases in relation to NGFR as dosage of Vem and Vem+Tram is increased

	0.1 uM	0.316uM
Vem	0.4004128	0.3994944
Vem + Tram	0.3860171	0.3554719



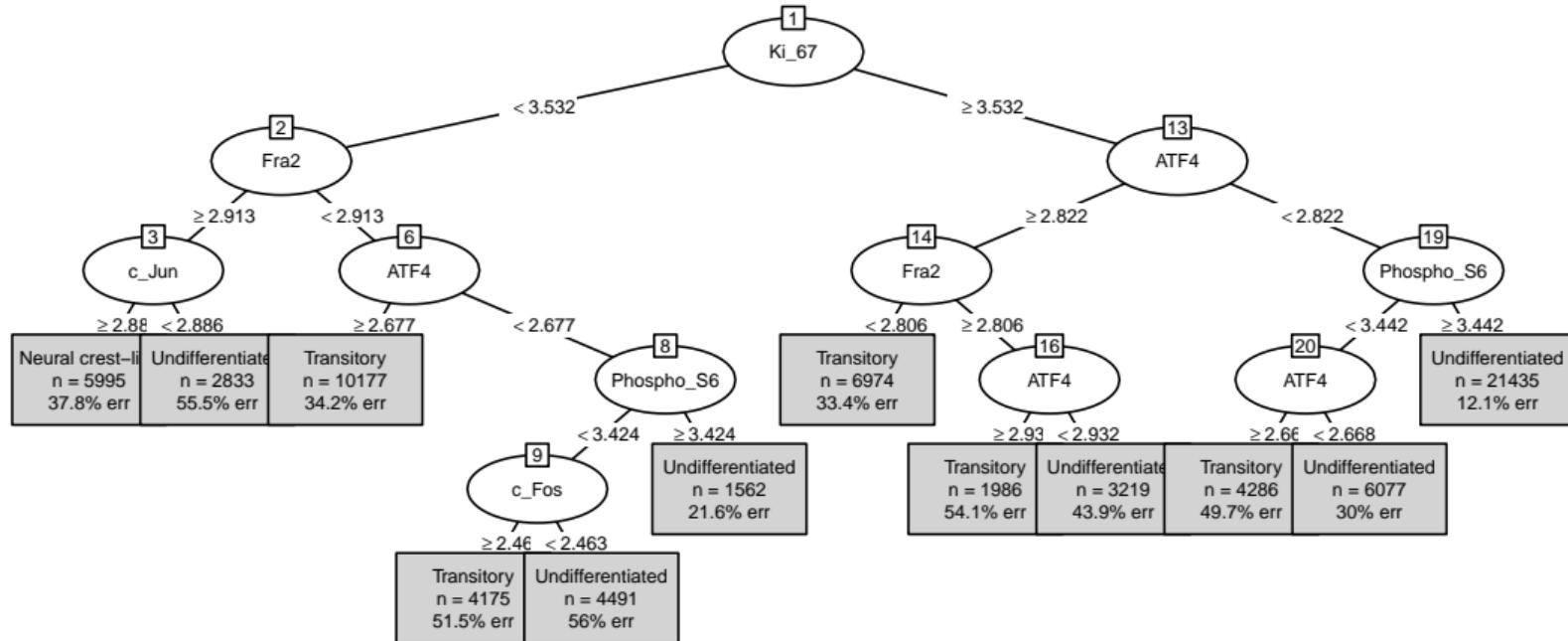
Correlation between SOX10 and AXL

- As shown by the table, there seems to be no correlation between SOX10 and AXL
- This means that they would not be good predictors of each other
- Protein level of AXL roughly stays the same in relation to SOX10 when dosage is increased for both drug treatments



	0.1 uM	0.316uM
Vem	0.0445903	0.0786711
Vem + Tram	0.0089317	0.0531577

Predicting Phenotype through Transcription Factors



Based on solely transcription factors, cells are most likely to be either Transitory or Undifferentiated

Predicting Phenotype Continued

Along with our previous observations, we can start to predict certain phenotypes

- The terminal nodes have high error so they may not be the best predictors
- However, certain transcription factors such as PhosphoS6, ATF4, and Ki67 may lead to an undifferentiated melanoma cell
- Similarly, transcription factors such as Ki67, ATF4, and Fra2 may lead to a transitory melanoma cell

	MiTFg	NGFR	SOX10	AXL
Undifferentiated	LOW	LOW	LOW	HIGH
Neural crest-like	LOW	HIGH	HIGH	HIGH
Transitory	HIGH	HIGH	HIGH	LOW
Melanocytic	HIGH	HIGH	LOW	LOW

Meta-analysis

Protein level in varying time and drug treatment:

- As time increases, levels of NGFR seems to increase while SOX10 stays roughly the same, and both AXL and MiTFg seem to decrease
- When Vem is used, the protein levels of MiTFg, SOX10, and AXL seems to decrease the most in comparison to Vem and Tram

Protein Correlation

- We want low dosage since the correlation between MiTFg and SOX10 is the strongest
- From previous analysis, MiTFg decrease with time and a high r value suggests a significant decrease in SOX10 which is desirable
- A low correlation would be a high risk in the case of NGFR and AXL, hence, we want a high correlation and thus low dosage in order to minimize risk

Meta-analysis continued

To encourage healthy homeostasis, we want to avoid certain transcription factors and instead encourage the presence of factors that result in undifferentiated cells

Transcription Factors

Phenotype	Transcription Factors
- Undifferentiated	- PhosphoS6, ATF4, and Ki_67 may lead to low levels of MiTFg, NGFR, and SOX10 and high levels of AXL
- Neural Crest-Like	- Ki_67, Fra2, and c_Jun can be used to predict low levels of MiTFg with high levels of NGFR, SOX10, and AXL
- Transitory	- Ki_67, ATF4, and Fra2 may lead to high levels of MiTFg, NGFR, and SOX10 and low levels of AXL
- Melanocytic	- Ki_67, Fra2, ATF4, or c_Fos could potentially lead to high levels of MiTFg and NGFR, and low levels of SOX10 and AXL

Conclusion

To keep the cell as undifferentiated and healthy, MiTFg, SOX10, and NGFR must be low while AXL is high, hence:

- We should use Vem with a relatively low dosage with an average amount of time to keep protein level around the same
- Since both SOX10 and MiTFg have no correlation in terms of dosage with AXL, only the dosage between AXL and NGFR matters
- We want to avoid treatments that increase levels of Ki_67, Fra2, ATF4, or c_Fos

Limitations and Assumptions:

- We conduct these statistical test assuming that only dosage, time, and drug affects protein level and no other confounding variable has underlying affects on the data
- Data and results can not be generalized or extrapolated to other carcinogenic cells since data collected is only about potentially carcinogenic melanoma cells