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Target Discovery Leveraging Multi-Omic Data and Comparability Assessment for Manufacturing Changes in CGT

Lira Pi

Data Strategy and Quantitative Sciences

BASS

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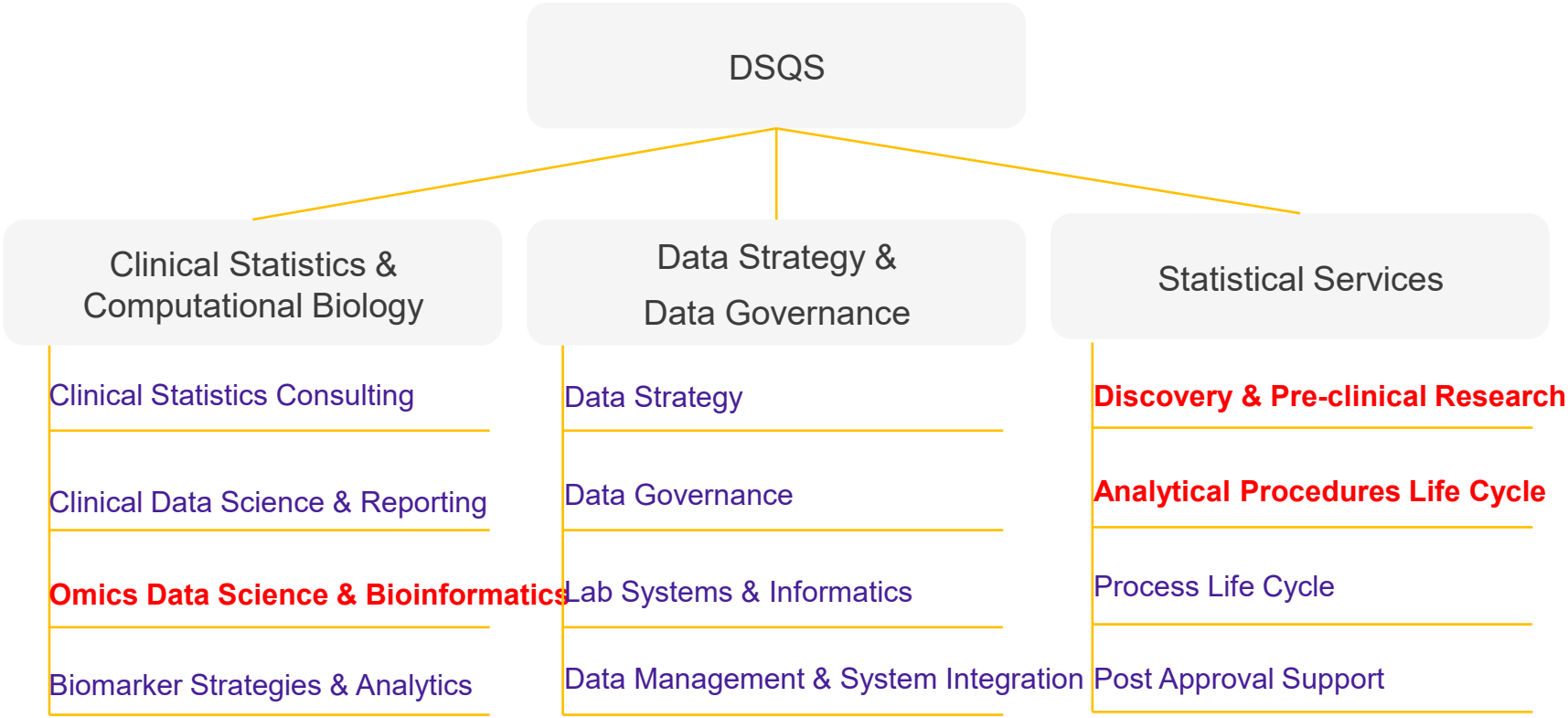
Data Strategy and Quantitative Sciences (DSQS)



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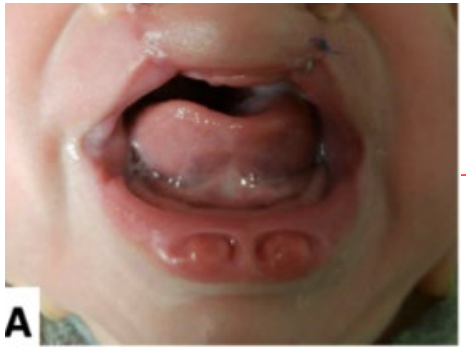
Target Discovery Leveraging Multi-Omics Data

Orofacial Cleft

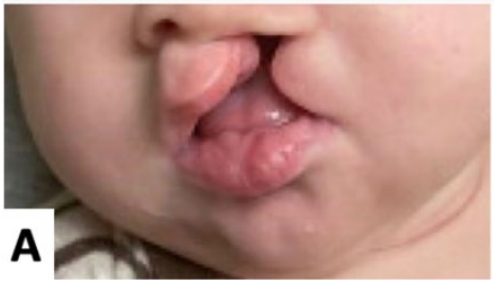
Knowledge gap: most of the heritable risk for non-syndromic orofacial cleft has not been assigned to a gene (e.g. non-coding functional SNP)

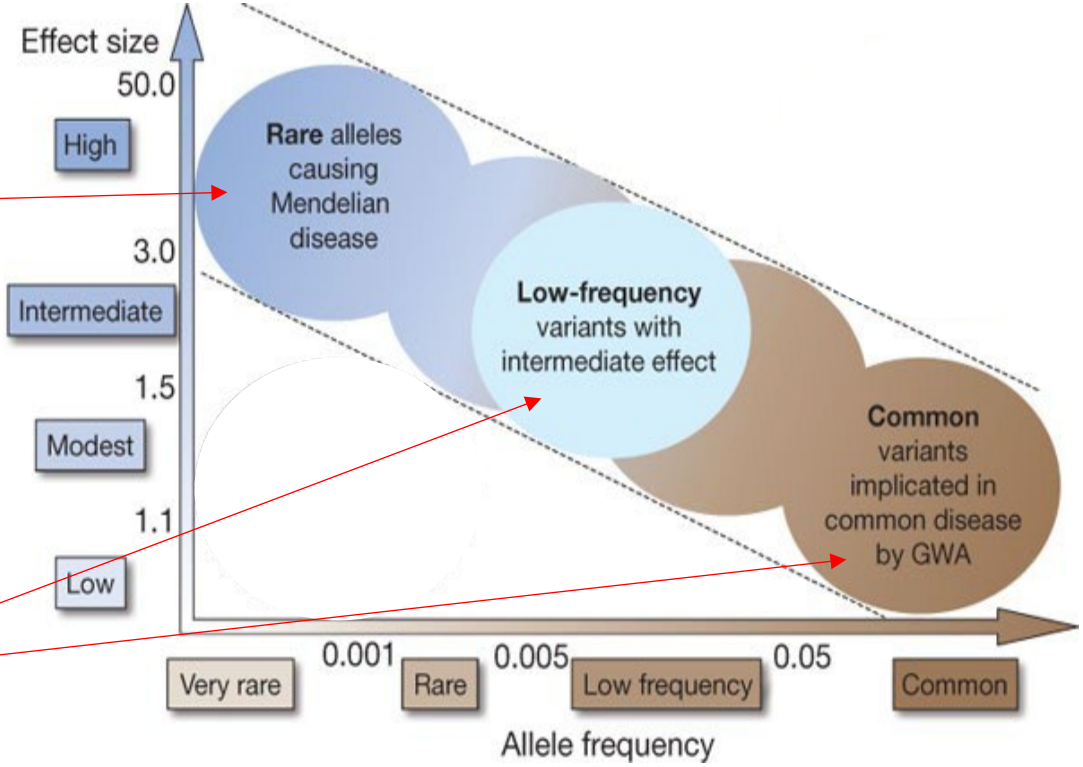
Syndromic



Van der Woude's Syndrome (IRF6)

Non-syndromic

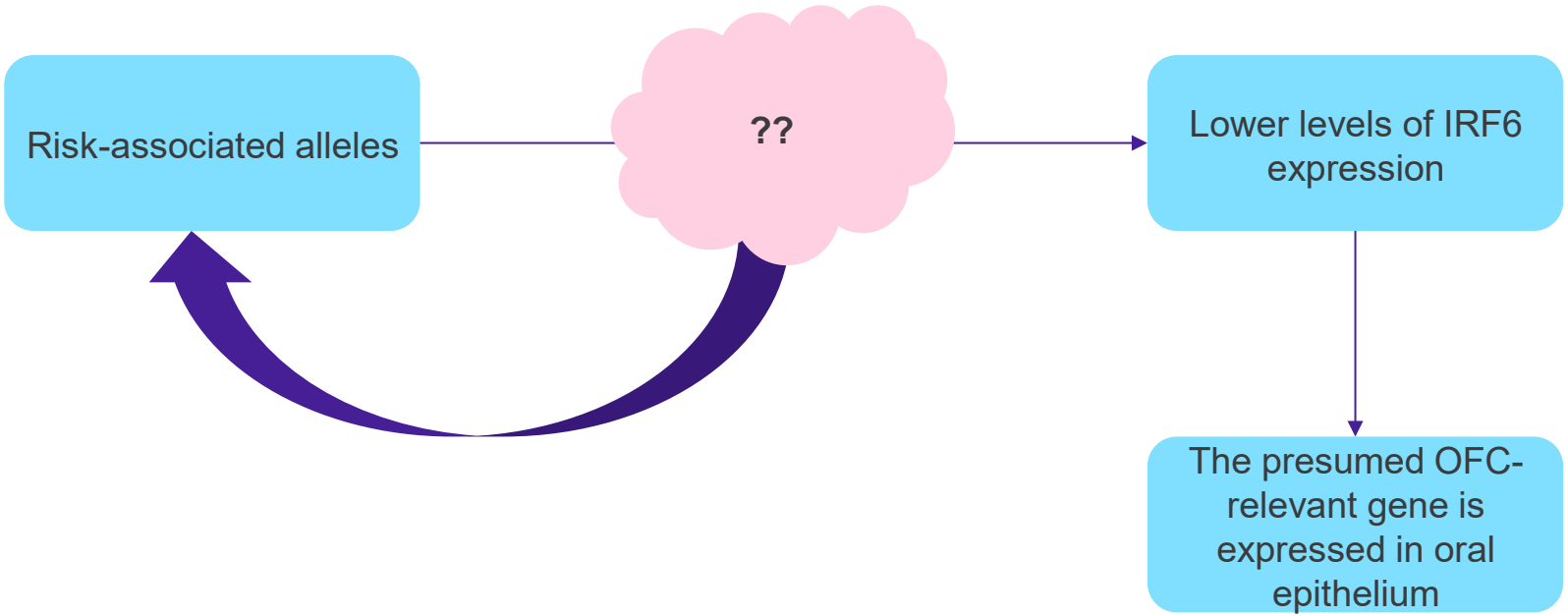




Marku L, Brooke SM, Koenig ZA. Orofacial Clefting in Van der Woude's Syndrome. Eplasty. 2023 Jan 9;23:QA1. PMID: 36793658; PMCID: PMC9891770.

Manolio TA, …, Visscher PM. Finding the missing heritability of complex diseases. Nature. 2009 Oct 8;461(7265):747-53. doi: 10.1038/nature08494. PMID: 19812666; PMCID: PMC2831613.

Mechanism Behind OFC



Discovery of Functional SNPs

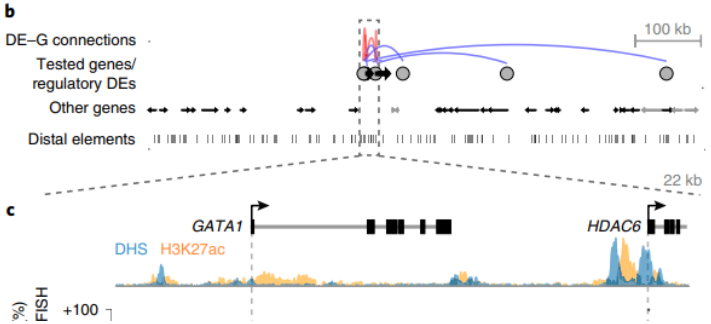
1. A massively parallel reporter assay (MPRA) was deployed to identify candidate functional SNPs among OFC-associated ones at IRF6/1q32 and several other loci where the OFC-risk-relevant gene is expressed in oral epithelium.

2. We tested 889 SNPs in an MPRA in an oral epithelium cell line and tested the validity of a subset of the MPRA results using traditional luciferase assays in the cell line.

3. For two promising SNPs near IRF6, we carried out extensive follow up experiments in oral epithelium cells derived from induced pluripotent stem cells whose genotype had been engineered.

4. These studies support the two SNPs as being functional, explain how they are functional, and show that they account for most of the heritable risk for OFC phenotypes attributed to this locus.

ABC (Activity-by-Contact) Model



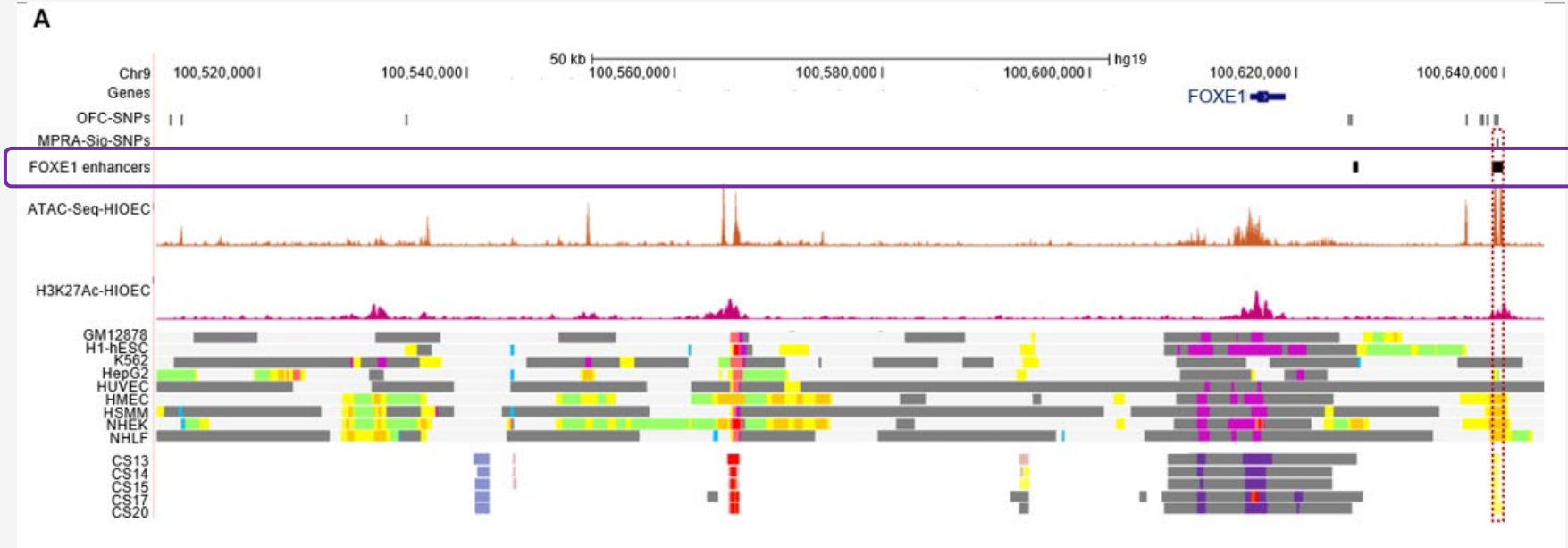
1. ABC model was applied to assign SNP-containing-enhancers to promoters (i.e., to predict enhancer-gene connections).

2. ABC model utilizes cell-type specific chromatin accessibility (ATAC-seq or DNase hypersensitivity) and chromatin activity (H3K27ac) and HiC data.

3. We used NHEK-specific publicly available datasets to calculate ABC score:
   - GSM733674 for H3K27ac
   - GSM736545 for DNase hypersensitivity
   - GSM958736 for RNA-seq and averaged HiC data from 10 cell types

*Fulco, C.P., Nasser, J., Jones, T.R. et al. Activity-by-contact model of enhancer–promoter regulation from thousands of CRISPR perturbations. Nat Genet **51**, 1664–1669 (2019). https://doi.org/10.1038/s41588-019-0538-0*

ABC Score

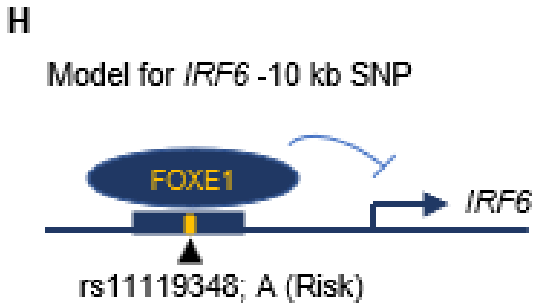


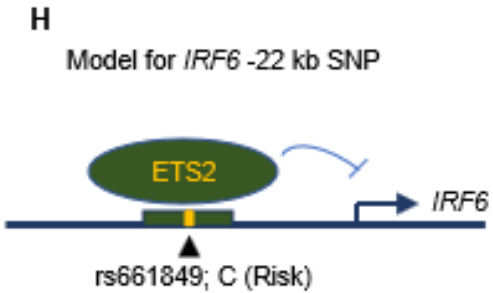
$$\text{ABC score}_{E,G} = \frac{A_E \times C_{E,G}}{\sum_{\text{all elements e within 5 Mb of } G} A_e \times C_{e,G}}$$

Kumari, Priyanka & Friedman, Ryan & Pi, Lira & Curtis, Sarah & Paraiso, Kitt & Visel, Axel & Rhea, Lindsey & Dunnwald, Martine & Patni, Anjali & Mar, Daniel & Bomsztyk, Karol & Mathieu, Julie & Ruohola-Baker, Hannele & Leslie, Elizabeth & White, Michael & Cohen, Barak & Cornell, Robert. (2024). Two non-coding variants associated with isolated orofacial cleft promote binding of transcriptional repressors FOXE1 or ETS2 and reduce expression of IRF6. 10.1101/2024.06.01.596914.

Summary: Two Common Variants Associated with Isolated OFC

Two common variants (rs11119348 and rs661849) were found be related with isolated OFC promote binding of transcriptional repressors FOXE1 and ETS2 and hence reduce expression of IRF6.





According to Fulco et al. (2019), two “Activity-by-Contact” pairs were identified as significantly functional with ABC scores > 0.02.

Kumari, Priyanka & Friedman, Ryan & Pi, Lira & Curtis, Sarah & Paraiso, Kitt & Visel, Axel & Rhea, Lindsey & Dunnwald, Martine & Patni, Anjali & Mar, Daniel & Bomsztyk, Karol & Mathieu, Julie & Ruohola-Baker, Hannele & Leslie, Elizabeth & White, Michael & Cohen, Barak & Cornell, Robert. (2024). Two non-coding variants associated with isolated orofacial cleft promote binding of transcriptional repressors FOXE1 or ETS2 and reduce expression of IRF6. 10.1101/2024.06.01.596914.

bioRxiv

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New Results

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Two non-coding variants associated with isolated orofacial cleft promote binding of transcriptional repressors FOXE1 or ETS2 and reduce expression of *IRF6*

Priyanka Kumari, Ryan Z. Friedman, Lira Pi, Sarah Curtis, Kitt Paraiso, Axel Visel, Lindsey Rhea, Martine Dunnwald, Anjali P. Patni, Daniel Mar, Karol Bomsztyk, Julie Mathieu, Hannele Ruohola-Baker, Elizabeth Leslie, Michael A. White, Barak A. Cohen, Robert A. Cornell

doi: https://doi.org/10.1101/2024.06.01.596914

It is publicly available in bioRxiv, which was submitted to Nature Communications.

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Comparability Assessment for Manufacturing Changes in CGT

Autologous cell therapy

1. Autologous = therapy that uses a patient's cells or tissues to treat various conditions, which are processed ex-vivo (i.e. outside the body) and reintroduced into the patient.
2. Cell therapy = cells are classified for their potential to transform into different cell type
3. Steps in Autologous cell therapy:
   - Cells are collected from the patient's body (e.g. through a blood sample or bone marrow biopsy).
   - Cells then are processed, being modified and expanded in laboratory according to the specific demands of the therapy.
   - The processed (modified) cells are returned to the patient through infusion, injection, or transplantation.
4. Autologous cell therapy can be applied to many conditions such as blood cancers, skin substitutes, wound healing, chronic inflammation.
5. Autologous cell therapy is less likely to cause immune responses than using donor cells since the cells come from the patient's own body.

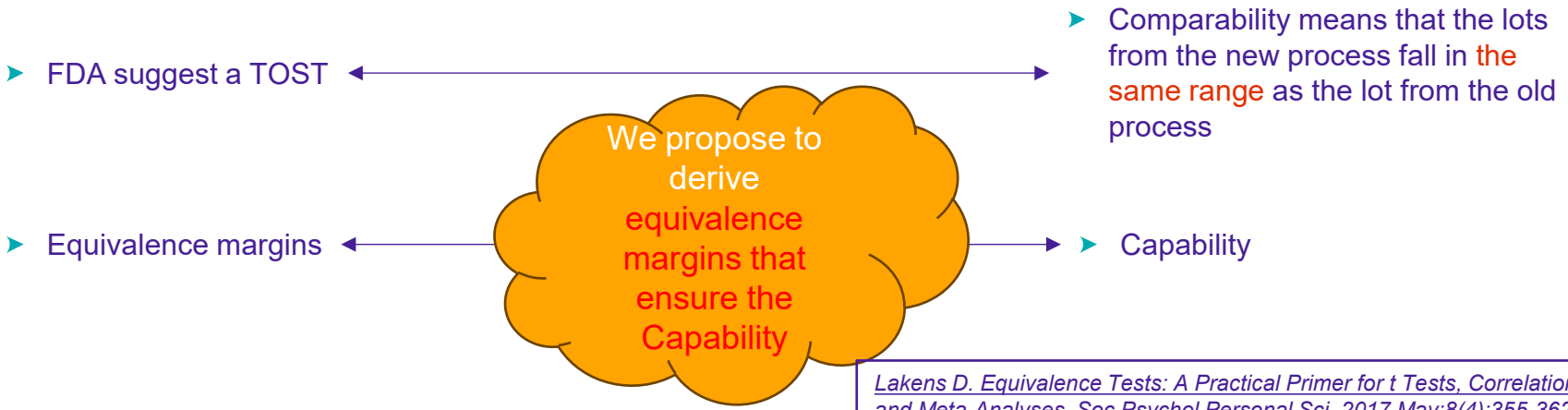
Farid SS, Jenkins MJ (2018). Bioprocesses for Cell Therapies In: Jagschies G, Lindskog E, Lacki K, Galliher P(eds) Biopharmaceutical Processing. Elsevier Ltd. https://doi.org/10.1016/C2014-0-01092-1

Manufacturing changes

1. Why doing manufacturing changes?
   - Improving product quality
   - Expanding product supply
   - Externalize manufacturing to a CDMO
   - Improving manufacturing efficiency
2. The change may adversely impact product quality → quality risk management
3. Challenges to do manufacturing changes in autologous therapies
   - Low sample size
   - High donor-to-donor variability
4. FDA drafted a guidance “Manufacturing changes and comparability for human cellular and gene therapy product (2023)”, *https://www.fda.gov/regulatory-information/search-fda-guidance-documents/manufacturing-changes-and-comparability-human-cellular-and-gene-therapy-products*
   - Comparability between pre-change and post-change is demonstrated by evidence that the change does not adversely affect product quality for the product

Manufacturers are being asked to demonstrate comparability

1. FDA guidance insists on risk assessment, selecting relevant quality attributes, analytical methods, acceptance criteria and statistical method
2. For autologous therapies where each lot is derived from a different donor,
   - Split-source design is recommended (cells of a single donor is divided into 2 pools of cells for each of the version of the process)
3. Guidance suggest performing a TOST with an equivalence margin defined before the study
4. In addition, the measurement also needs to meet the in-process and relevant acceptance criteria

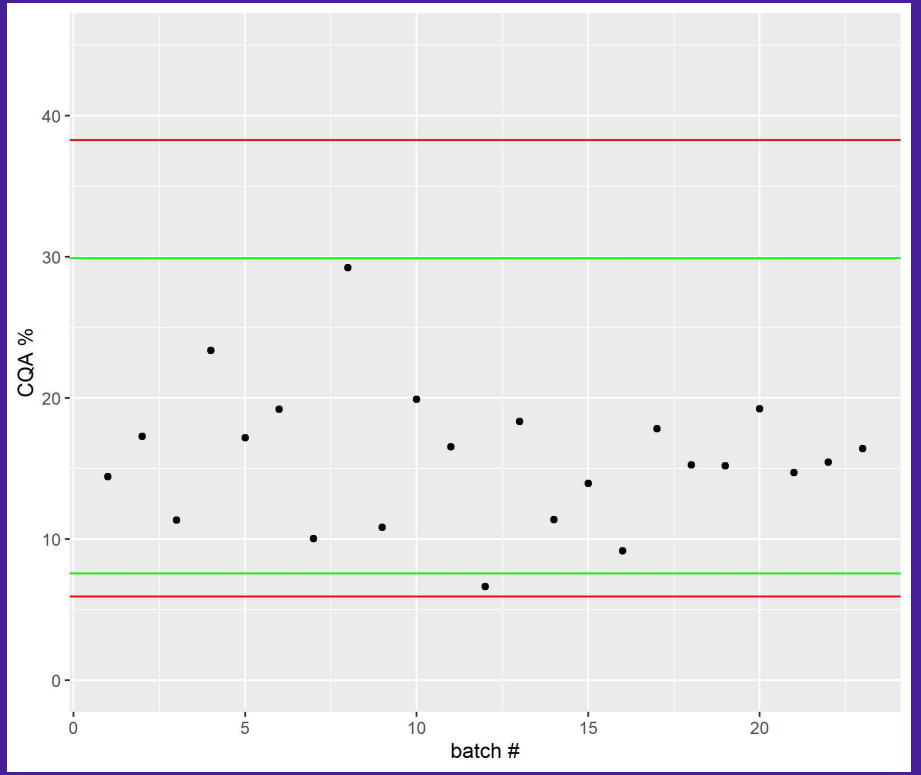


Lakens D. Equivalence Tests: A Practical Primer for t Tests, Correlations, and Meta-Analyses. Soc Psychol Personal Sci. 2017 May;8(4):355-362. doi: 10.1177/1948550617697177. Epub 2017 May 5. PMID: 28736600; PMCID: PMC5502906.

Historical data of old process

Capability is the probability that the new process fall within limits of the old process (= PI)

Example of data that were accumulated before the change of manufacturing process.
The 2 intervals represents the prediction interval at 95% and 99% (assuming a log normal distribution)



Framework of Simulation

Consider the transformation in the calculation wherever it is needed.

1. Generate data for the old and new process
   - Assume μ equals the mean of historical data
   - Generate $true\ donors \sim Normal(\mu, \sigma_{donor})$. We usually don't know between donor variability, but it is the main component and should likely represent 1/3 or 1/2 of the variability of the historical data.
   - True donor values are replicated for each of the process
   - For the new process, add a hypothesized shift/ratio between the two processes
2. Calculate 90% confidence on the difference between the 2 process (or ratios if this is log data)
3. Calculate capability of the new process to be in the PI of the old process
4. Repeat steps 1 to 3 a lot of time varying the hypothesized shift/ratio
5. We obtain a plot of capability against the bound of the confidence intervals

Results

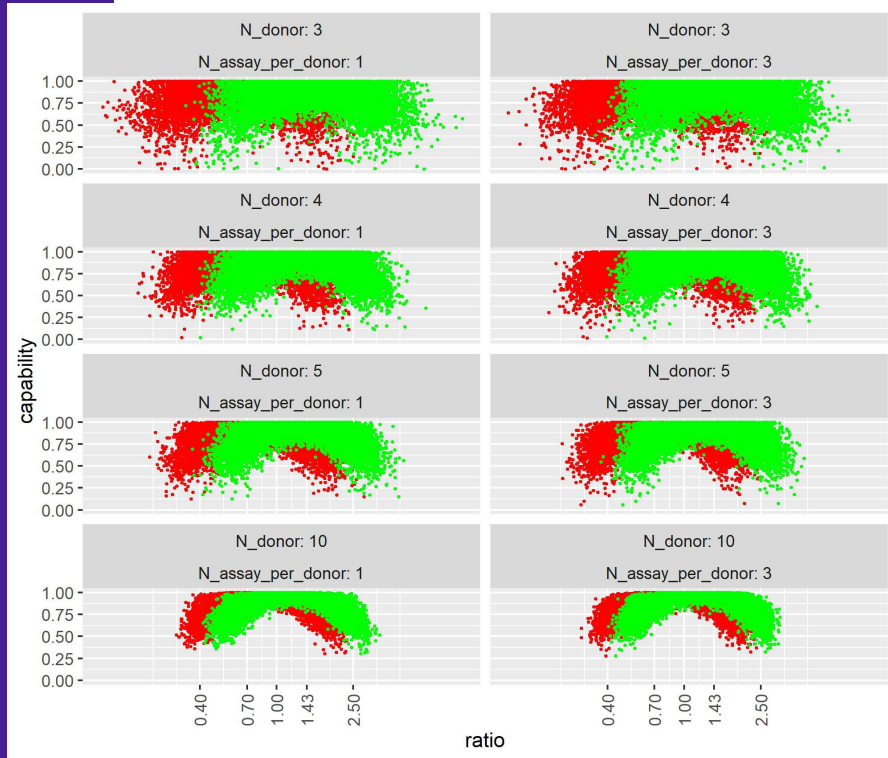
Were varied

- Number of donors
- Number of measurements per donor

The red and green colors represent respectively the lower and upper bounds of the confidence interval.

We can read from the plot what is the lower and upper bound of the confidence interval ensuring a minimum capability.

Due to the spread of the data, we use a logistic regression (details on next slides).



Logistic regression on simulation results

1. Assuming we want a minimum capability of 80%
2. The column containing the capability results is converted into 0 or 1 (if > 80%, 1, else 0)
3. Fit two logistic regressions where the predictor is

- Intercept + Lower bound of CI + (Lower bound of CI)^2 = $\hat{\beta}_L C_L$
- Intercept + Upper bound of CI + (Upper bound of CI)^2 = $\hat{\beta}_U C_U$

4. Solve the 2nd order equation to solve what are the Lower/Upper bound of CI that ensure a minimum probability (80%) to reach 80% capability
5. Get the lower and upper bound of CI ensuring 80% probability to get 80% minimum capability
6. Criteria doesn't depend on the number of donor or the assay variability
7. The test will be to calculate the CI on the ratio between the 2 process. If this is included in [0.50, 2.00], the comparability is demonstrated, because we have 80% probability that the capability of the new process is at least 80% to be in the PI of the old process.

$$\Pr(capability \geq 0.80) \geq 0.80$$

$$\log(0.25) = \hat{\beta}_L C_L = \hat{\beta}_U C_U$$

N_donor	N_assay_per_donor	criteria_lower	criteria_upper
3	1	0.54	1.82
3	3	0.52	1.91
4	1	0.49	1.95
4	3	0.49	2.00
5	1	0.49	2.04
5	3	0.49	2.04
10	1	0.49	2.00
10	3	0.49	2.00

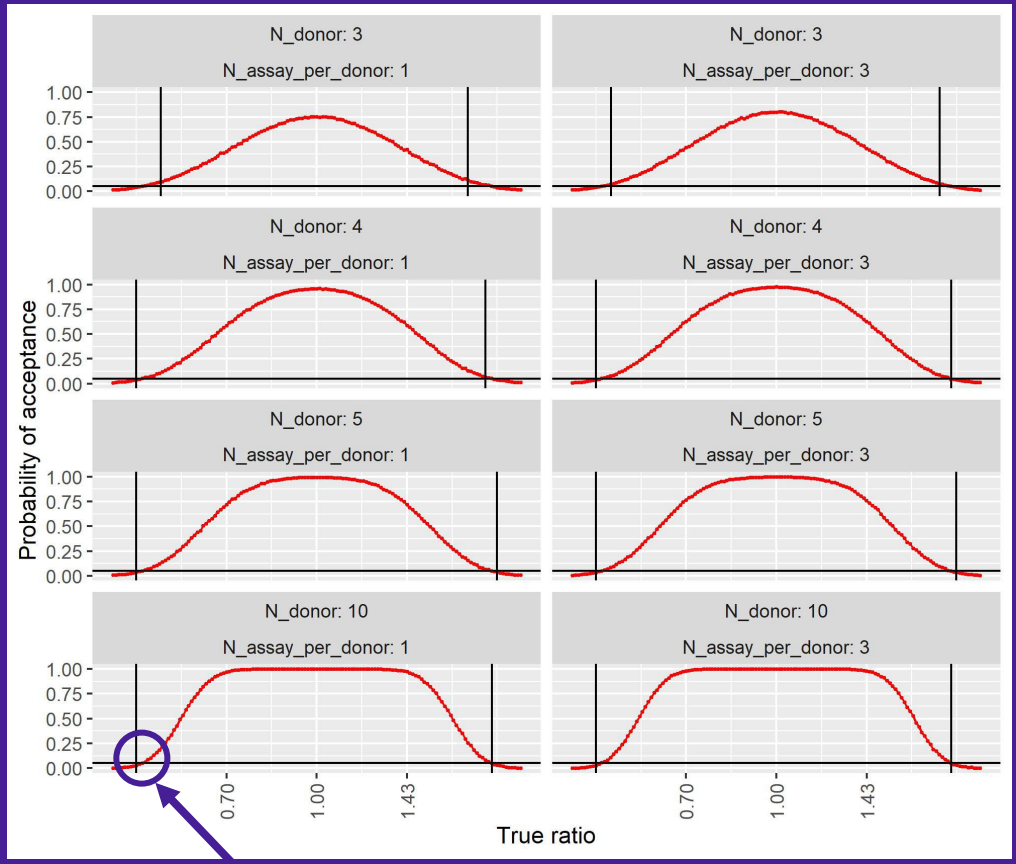
To evaluate the sample size

1. Let's draw operating curves
2. Generate data for the old and new process
   - Assume μ equals respectively the mean of historical data
   - Generate $true\ donors \sim Normal(\mu, \sigma_{donor})$. We usually don't know between donor variability, but it is the main component and should likely represent 1/3 or 1/2 of the variability of the historical data.
   - True donor values are replicated for each of the process
   - For the new process, add a hypothesized shift/ratio between the two processes
3. Calculate 90% confidence on the difference between the 2 process (or ratios if this is log data)
4. If this is [0.50 , 2.00], this is PASS otherwise fail.
5. Repeat steps 1 to 3 a high number of times to calculate the probability of acceptance for a given value of the hypothesized shift/ratio
6. Repeat steps 1 to 4 with varying hypothesized shift/ratio to get operating curves

Results of operating curves

1. X axis is the hypothesized ratio
2. Y axis is the probability of acceptance
3. Vertical lines are criteria on the CI on the ratio
4. When the sample size or the number of measurement per donor increase, probability of acceptance are steeper
5. Curves cross the criteria at 5% probability of acceptance in all scenarios

- Criteria is on the CI
- X axis is not the CI. This is the assumed mean ratio. It is not possible to assume a true CI
- 90% CI is used. On the lower end, 5% of CI will not contain the true ratio. This is why the curve crosses at 5% probability



5% probability of acceptance

Conclusions

We derived a criteria

- that complies with the FDA draft guidance
- And that ensure capability of the new process, the probability falling in the acceptable range of the old process

- Bayesian statistics allows to derive future capability given actual data
- Back calculation allows to derive acceptance limits that control risk

Thank you

Lira Pi (lira.pi@pharmalex.com)

Associate Director
Cencora - PharmaLex
Data Strategy & Quantitative Sciences