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Together we are beating cancer

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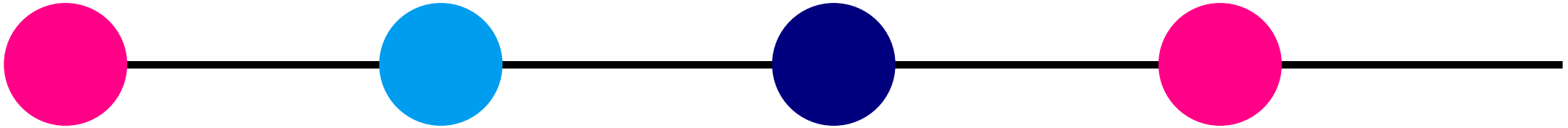
21st February 2025

Linear regression models

Fixed-effects models

Process flow



Stage 1

Effects and regression models

Stage 2

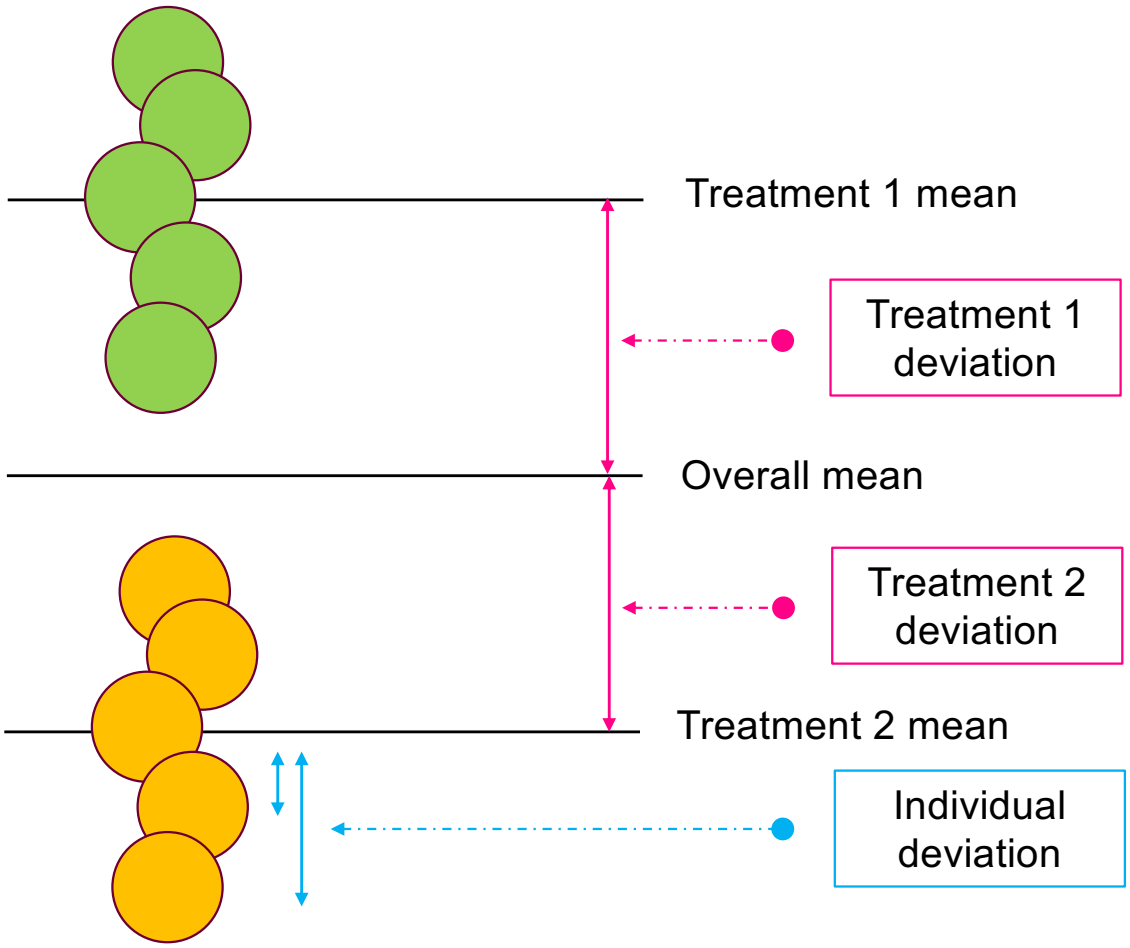
Analysis of variance (ANOVA)

Stage 3

Simple regression model

Stage 4

Multivariable regression model



Qualitative predictor

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Analysis of variance (ANOVA)

Definition and classification

10.00 -10.20 am

Together we are beating cancer

Fisher's one-way ANOVA

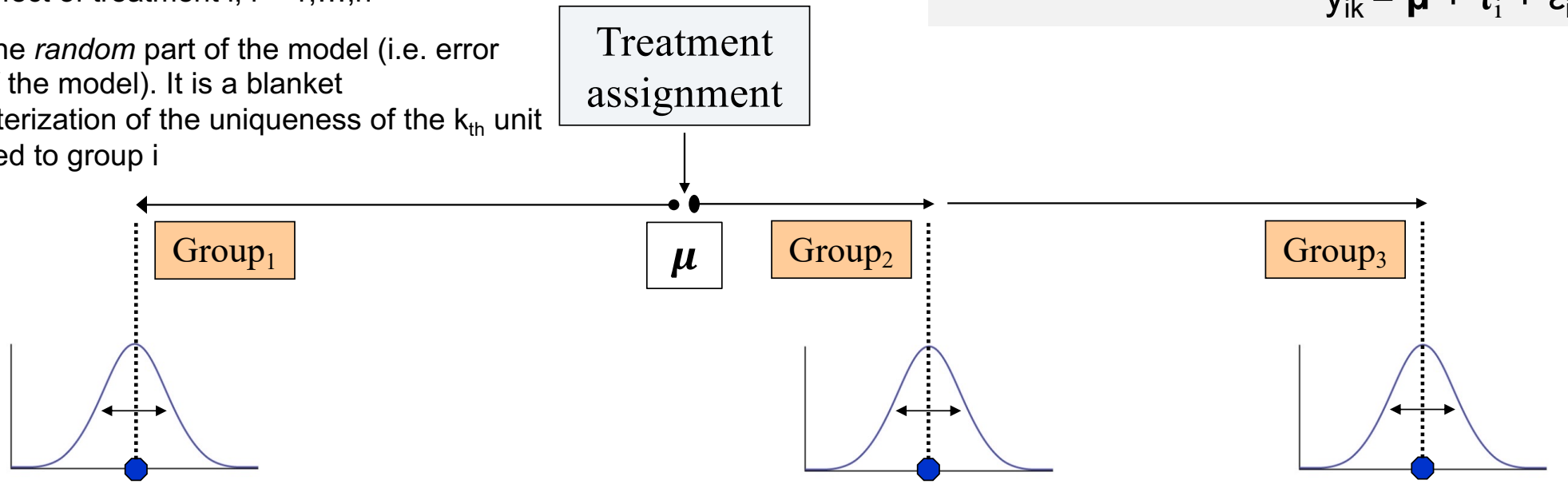
1. The unit k (e.g. mouse), k = 1,…,u_i; N = Σ_i u_i

2. τ_i: effect of treatment i, i = 1,…,n

3. ε_{ik}: the *random* part of the model (i.e. error term of the model). It is a blanket characterization of the uniqueness of the k_{th} unit assigned to group i

Equation of the statistical model:

$$y_{ik} = \mu + \tau_i + \varepsilon_{ik}$$



Assumptions of ANOVA (ANalysis Of VAriance) models are the following:

- The effect of each treatment level i is additive on μ (i.e. population mean) parameter
- ε_{ik} is assumed to be independent of one another and normally distributed with mean = 0 and common standard deviation = σ

Fisher's one-way ANOVA

Hypothesis to test: $\tau_1 = \ldots = \tau_n = 0$

Test statistic:

Source of variation	**Sum of Squares**	**Degrees of freedom**	**Mean Squares**	**$F_{df1,df2}$**	**p-value**
Treatment	SSB = $\Sigma_i\, u_i\, (m_i - M)^2$	$df_1 = n - 1$	MSB = SSB / df_1	MSB / MSE	0.023
Residuals	SSE = $\Sigma_i \Sigma_k\, (y_{ik} - m_i)^2$	$df_2 = N - n$	MSE = SSE / df_2		
Total	SST = SSB + SSE				

Legend: m_i is the sample mean of group i. M is the overall mean response

Note: the ANOVA divides the total variation in the response into parts.

R implementation		
Step	**Aim**	**R function**
1	We should fit our data to the ANOVA model	fitModel = lm(*Response* ~ *Treatment*, data=*dSet*)
2	We can get R to produce an ANOVA table	anova(fitModel)

Fisher's two-way ANOVA

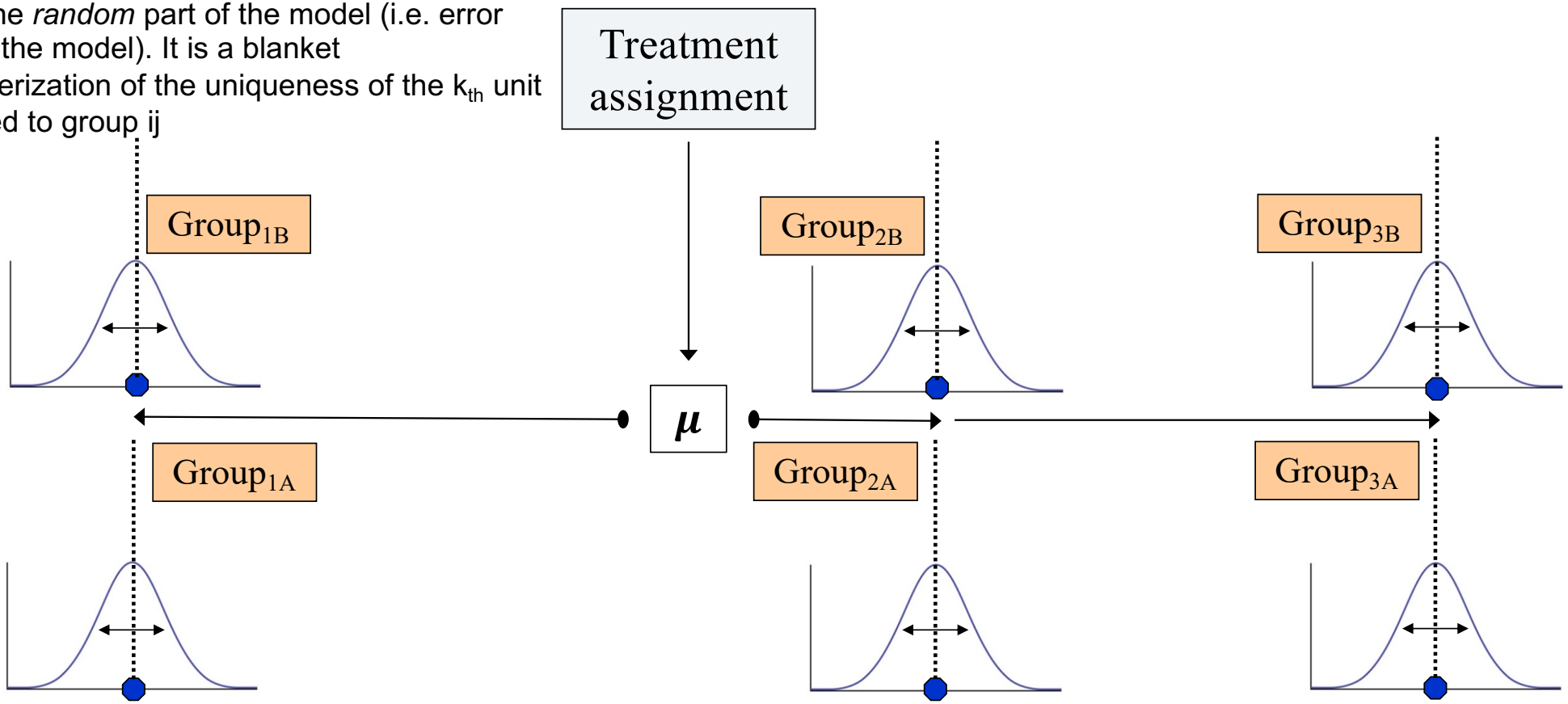
1. The unit k (e.g. mouse), k = 1,…,u_{ij}; N = Σ_{ij} u_{ij}

2. τ_i: effect of treatment i, i = 1,…,n; η_j: effect of treatment j, j = 1,…,r

3. ε_{ijk}: the *random* part of the model (i.e. error term of the model). It is a blanket characterization of the uniqueness of the k_{th} unit assigned to group ij

Equation of the statistical model:

$$y_{ijk} = \mu + \tau_i + \eta_j + \varepsilon_{ijk}$$



Fisher's two-way ANOVA

Hypothesis to test n.1: $\tau_1 = \ldots = \tau_n = 0$

Hypothesis to test n.2: $\eta_1 = \ldots = \eta_r = 0$

Test statistic:

Source of variation	**Sum of Squares**	**Degrees of freedom**	**Mean Squares**	**$F_{df1,df2}$**	**p-value**
Treatment τ	$SSB_\tau = \Sigma_i\, u_i\, (m_i - M)^2$	$df1_\tau = n - 1$	$MSB_\tau = SSB_\tau / df1_\tau$	MSB_τ / MSE	0.023
Treatment η	$SSB_\eta = \Sigma_j\, u_i\, (m_j - M)^2$	$df1_\eta = r - 1$	$MSB_\eta = SSB_\eta / df1_\eta$	MSB_η / MSE	0.150
Residuals	$SSE = \Sigma_{ij} \Sigma_k\, (y_{ijk} - m_{ij})^2$	$df_2 = N - (n \cdot r)$	$MSE = SSE / df_2$		
Total	$SST = SSB_\tau + SSB_\eta\, SSE$				

Note: the ANOVA divides the total variation in the response into parts.

R implementation		
Step	**Aim**	**R function**
1	We should fit our data to the ANOVA model	fitModel = lm(*Response* ~ $Treat_\tau$ + $Treat_\eta$, data=*dSet*)
2	We can get R to produce an ANOVA table	anova(fitModel)

Fisher's two-way ANOVA with interaction

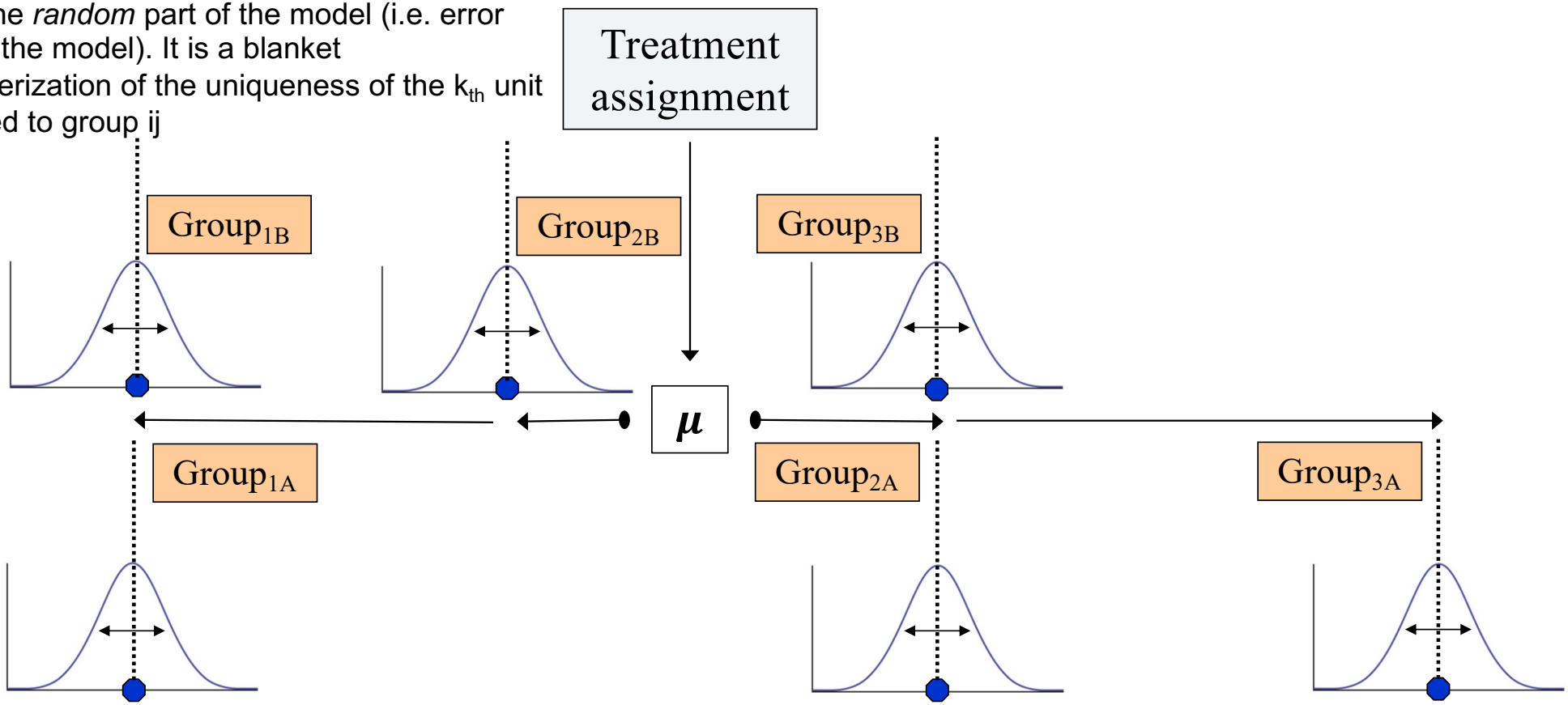
1. The unit k (e.g. mouse), k = 1,…,u_{ij}; N = Σ_{ij} u_{ij}

2. τ_i: effect of treatment i, i = 1,…,n; η_j: effect of treatment j, j = 1,…,r

3. ε_{ijk}: the *random* part of the model (i.e. error term of the model). It is a blanket characterization of the uniqueness of the k_{th} unit assigned to group ij

Equation of the statistical model:

$$y_{ijk} = \mu + \tau_i + \eta_j + \tau_i{:}\eta_j + \varepsilon_{ijk}$$



Fisher's two-way ANOVA with interaction

Hypothesis to test n.1: $\tau_1 = \ldots = \tau_n = 0$	Hypothesis to test n.2: $\eta_1 = \ldots = \eta_r = 0$	Hypothesis to test n.3: $\tau{:}\eta = 0$

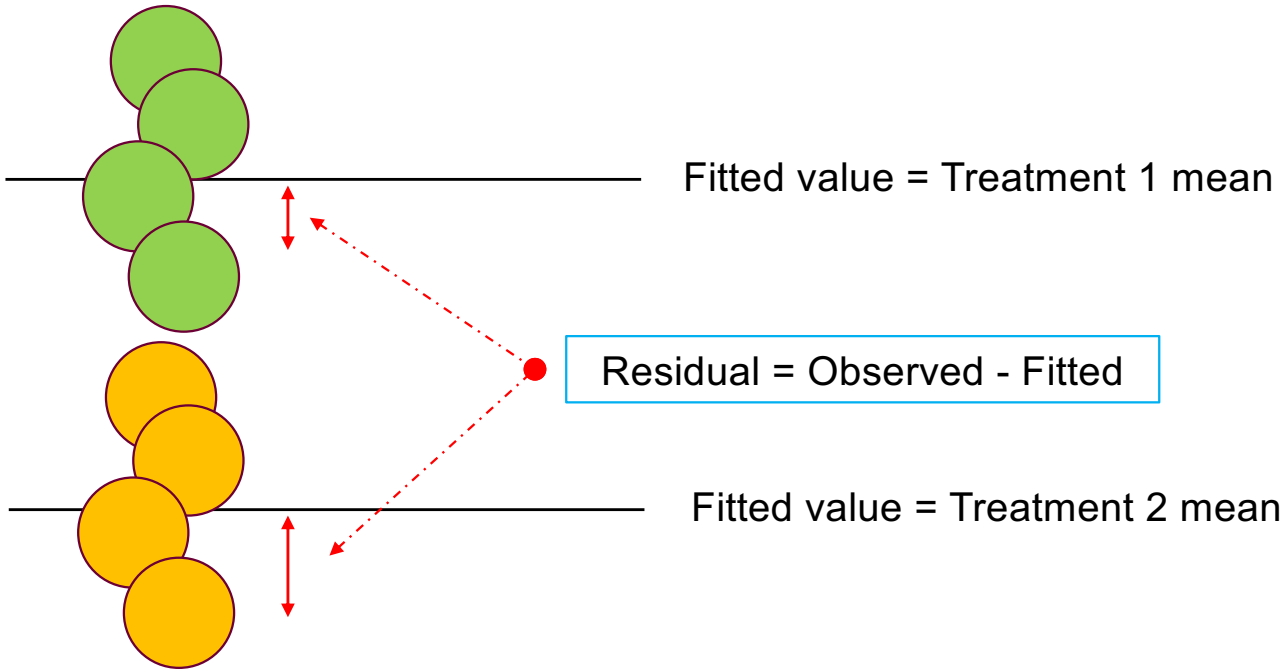
Test statistic:

Source of variation	**Sum of Squares**	**Degrees of freedom**	**Mean Squares**	**$F_{df1,df2}$**	**p-value**
Treatment τ	$SSB_\tau = \Sigma_i\, u_i\, (m_i - M)^2$	$df1_\tau = n - 1$	$MSB_\tau = SSB_\tau / df1_\tau$	MSB_τ / MSE	0.023
Treatment η	$SSB_\eta = \Sigma_j\, u_i\, (m_j - M)^2$	$df1_\eta = r - 1$	$MSB_\eta = SSB_\eta / df1_\eta$	MSB_η / MSE	0.150
Interaction $\tau{:}\eta$	$SSB_{\tau:\eta} = \Sigma_{jj}\, u_{ij}\, (m_{ij} - m_j - m_i + M)^2$	$df1_{\tau:\eta} = (n - 1) \cdot (r - 1)$	$MSB_{\tau:\eta}\, SSB_{\tau:\eta} / df1_{\tau:\eta}$	$MSB_{\tau:\eta}$ / MSE	0.401
Residuals	$SSE = \Sigma_{ij}\, \Sigma_k\, (y_{ijk} - m_{ij})^2$	$df_2 = N - (n \cdot r)$	$MSE = SSE / df_2$		
Total	$SST = SSB_\tau + SSB_\eta + SSB_{\tau:\eta} + SSE$				

Note: the ANOVA divides the total variation in the response into parts.

R implementation		
Step	**Aim**	**R function**
1	We should fit our data to the ANOVA model	fitModel = lm(Response ~ $Treat_\tau$ * $Treat_\eta$, data=*dSet*)
2	We can get R to produce an ANOVA table	anova(fitModel)

Diagnostics: residuals



The (raw) residuals are equal to the difference between the observations and the corresponding fitted values.

R implementation		
Step	**Aim**	**R function**
1	We should fit our data to the ANOVA model	fittedModel = lm(*Response* ~ *Predictor*, data=*dSet*)
2	We want to obtain the *residuals* of the model	*dSet*$resid = resid(fittedModel)

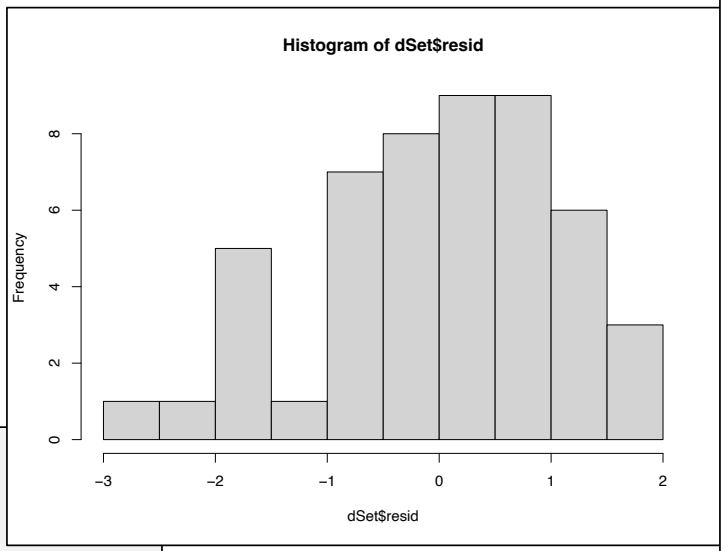
Diagnostics: residuals

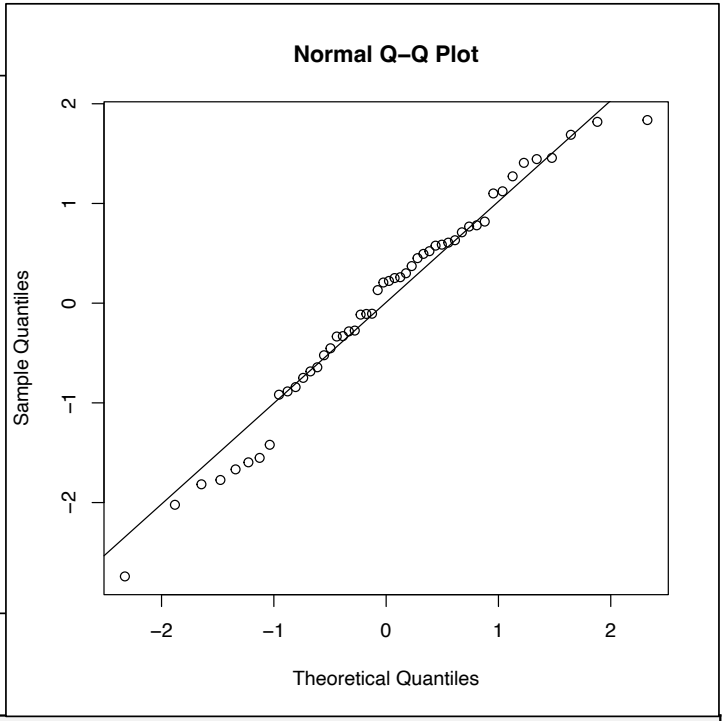
Shapiro-Wilk normality test

data: dSet$resid
W = 0.97324, p-value = 0.3119

Bartlett test of homogeneity of variances

data: resid by Predictor
Bartlett's K-squared = 1.5374, df = 1, p-value = 0.215





R implementation

Step	Aim	Tool	R function
1	We should plot the *residuals*	Histogram	hist(*dSet*$resid)
		Q-Q plot	qqnorm(*dSet*$resid); qqline(*dSet*$resid)
2	We could test the assumptions	Shapiro-Wilk *normality* test	shapiro.test(*dSet*$resid)
		Bartlett's *homoscedasticity* test	bartlett.test(resid ~ *Predictor*, data = *dSet*)

Diagnostics: residuals

Equation of the statistical model:

$$y_{ijk} = \mu + \tau_i + \eta_j + \tau_i{:}\eta_j + \varepsilon_{ijk}$$

Assumptions of normality and homoscedasticity **must be satisfied** by residuals of single treatment group and **combined** treatment groups (e.g. **pooled** residuals of $Group_{1A}$, $Group_{3A}$ and $Group_{3B}$).
Pooled residuals should be examined in the diagnostic analysis.

Source of problems and possible solutions

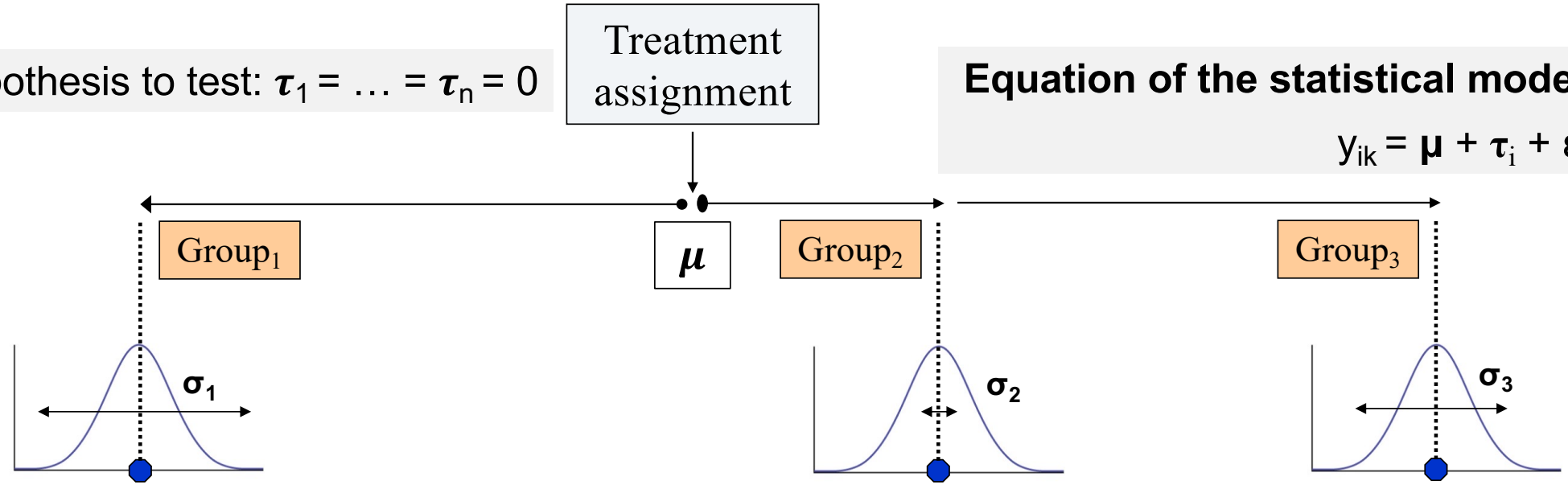
Solution	Normality	Unequal variance	Outliers
Welch's one-way ANOVA		✓	
Weighting		✓	
Distribution-free methods ⊙	✓	✓	✓
Data transformation	✓	✓	✓

⊙ e.g. Kruskal-Wallis test (i.e. one-way ANOVA on ranks)

Welch's one-way ANOVA

The Welch version of one-way ANOVA do not assume that all the groups are sampled from populations with equal variances.

Hypothesis to test: $\tau_1 = \ldots = \tau_n = 0$



Equation of the statistical model:

$$y_{ik} = \mu + \tau_i + \varepsilon_{ik}$$

Assumptions of Welch's one-way ANOVA models are the following:

- The effect of each factor is additive on μ (i.e. population mean) parameter
- ε_{ik} is assumed to be independent of one another and normally distributed with mean = 0. **Standard deviation could be different between groups: $\sigma_i \neq \sigma_j$, $i \neq j$.**

Weighted least squares


```
> library(nlme) # Load the nlme package
> fittedModel = gls(Response ~ Predictor, weight = varIdent(form= ~1 | Predictor) , data = dSet)
    # Modeling variance as function of the predictor
> summary(fittedModel) # Output
```

Output

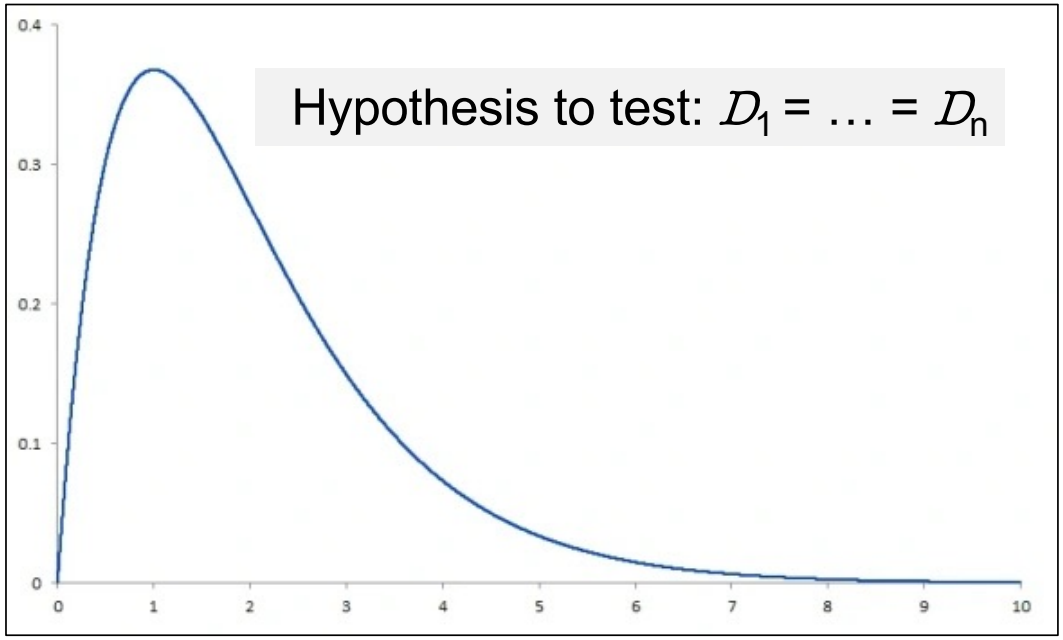
```
Variance function:
 Structure: Different standard deviations per stratum
 Formula: ~1 | gender
 Parameter estimates:
      F       M
1.0000000 0.7786179

Coefficients:
             Value Std.Error  t-value p-value
(Intercept) 147.4729 3.725061 39.58938  0.0000
gender M      4.9198 5.097216  0.96519  0.3368
```

Note: gls() function fits a linear model using generalized least squares.

Kruskal-Wallis test

The Kruskal-Wallis test (i.e. one-way ANOVA on ranks) works on ranks. It tests whether samples originate from the same distribution.



10.2	24.7	33.2	..	96.4	99.9

Replacement of data by ranks

1	2	3	..	N-1	N

Assumptions of Kruskal-Wallis test are the following:

- We only assume that the observations in the data set are independent of one another.

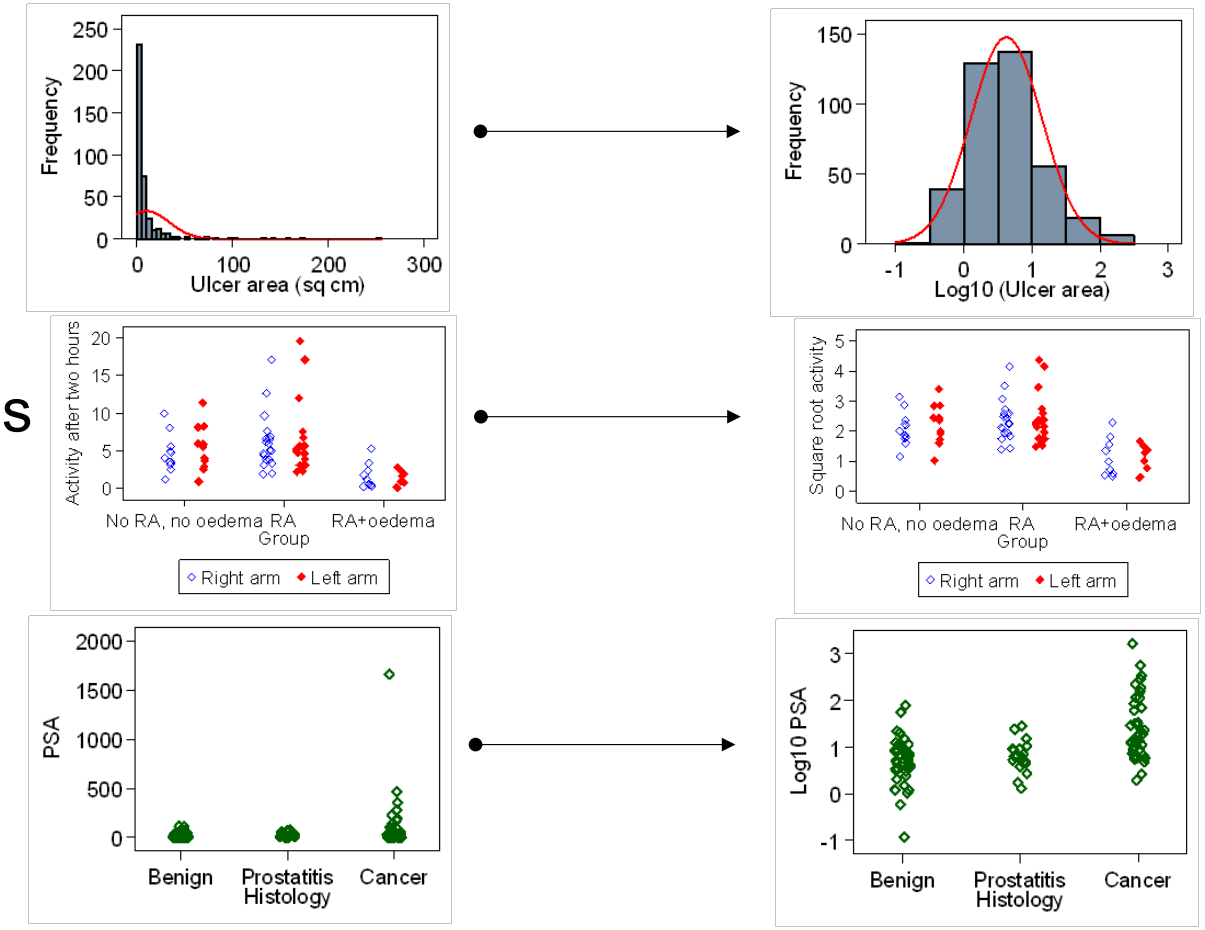
R functions

R implementation		
Test	**R**	
Welch's one-way ANOVA	Function	• oneway.test(*Response ~ Predictor*, data = *dSet*, var.equal = FALSE)
	Output	One-way analysis of means (not assuming equal variances) data: Response and Predictor F = 118.34, num df = 1.000, denom df = 45.143, p-value = 3.342e-14
Weighted least square	Function	• fittedModel <- gls(*Response ~ Predictor*, weights = varIdent(form= ~ 1 | *Predictor*), data = *dSet*) • summary(fittedModel)
	Output	Variance function: Structure: Different standard deviations per stratum Formula: ~1 | Predictor Parameter estimates: 1 2 1.000000 1.293192 Coefficients: Value Std.Error t-value p-value (Intercept) -0.001177 0.1890228 -0.006228 0.9951 Predictor 3.361487 0.3090014 10.878548 0.0000
Kruskal-Wallis	Function	• kruskal.test(*Response ~ Predictor*, data = *dSet*)
	Output	Kruskal-Wallis rank sum test data: Response by Predictor Kruskal-Wallis chi-squared = 34.222, df = 1, p-value = 4.917e-09

Data transformation

We can transform the data mathematically…

- to make them fit the normality more closely
- to obtain more similar variances
- to handle outliers



Data transformation

Common and useful transformations of the response variable:

1. the logarithm ($y_i > 0$, i=1,…n)
2. the square root ($y_i \geq 0$, i=1,…n)
3. the square power ($y_i \geq 0$, i=1,…n)
4. the ranks (e.g. Welch's one-way ANOVA on ranks)

https://bioinformatics-core-shared-training.github.io/Fixed-and-Mixed-effects-models/