Statistical Analysis Plan

TRIAL FULL TITLE	Study of Tolerance to Oral Peanut: Determining the efficacy of oral immunotherapy in peanut allergy
SAP VERSION	1.0
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2 Abbreviations and Definitions

AE	Adverse event
AUC	Area under curve
CRF	Case report form
DBPCFC	Double blind placebo controlled food challenge
IgE	Immunoglobulin E
LOAEL	Lowest observed adverse effect level
NOAEL	No observed adverse effect level
OIT	Oral Immunotherapy
QoL	Quality of life
SAP	Statistical analysis plan
SPT	Skin prick test
SUSAR	Suspected unexpected serious adverse reaction
WAO	World Allergy Organisation

3 Introduction

The aim is to develop a new treatment for a common, serious and currently untreatable condition. We propose a definitive study of the efficacy of peanut oral immunotherapy (OIT) as a treatment for peanut allergy. Immunological mechanisms will be studied. We have conducted a pilot which demonstrated proof of concept; the present study includes larger numbers and a control group, with power to detect outcome at the 0.05 significance level. There will be two inter-dependent work packages. Package 1 will be a randomized comparison of intervention versus the current best management. Package 2 will confirm efficacy in the waiting list group when subsequently treated and allow an estimate of the overall success rate to within 10%.

4 Study Objectives and Endpoints

4.1 Study Objectives

The overarching objective is to determine efficacy of oral immunotherapy in peanut allergy. We will determine whether the planned intervention is successful in the intervention group compared to control (package 1), and whether it is successful when offered to the control group (package 2). Other objectives include identification of immunological changes over time, and improvement in quality of life scores.

4.2 Endpoints

4.2.1 Primary Endpoint

Incidence of desensitisation to peanut at the end of six months.

4.2.2 Secondary Endpoints

- Incidence of desensitisation to peanut in waiting list patients after receiving the active intervention (end of phase 2)
- Incidence of response to treatment(end of phase 1 (intervention group) and end of phase 2 (waiting list group))
- Fold and absolute increase in threshold (maximum tolerated peanut protein (mg))
- Change in quality of life (QoL) scores from baseline to the end of phase 1
- Change in QoL scores before and after immunotherapy (Baseline to phase 1(intervention group) or phase 1 to phase 2 (waiting list group).
- Change in immunological outcomes (Basophil histamine release, Peanut IgE, Total IgE, and skin prick test diameter (SPT))
- Change in severity of symptoms (World Allergy Organisation (WAO) score) from baseline to the end of phase 1

4.3 Derived variables

- Desensitisation to peanut – able to ingest 1400mg peanut protein without reacting
- Response to treatment – able to ingest 800mg protein without reacting, after immunotherapy

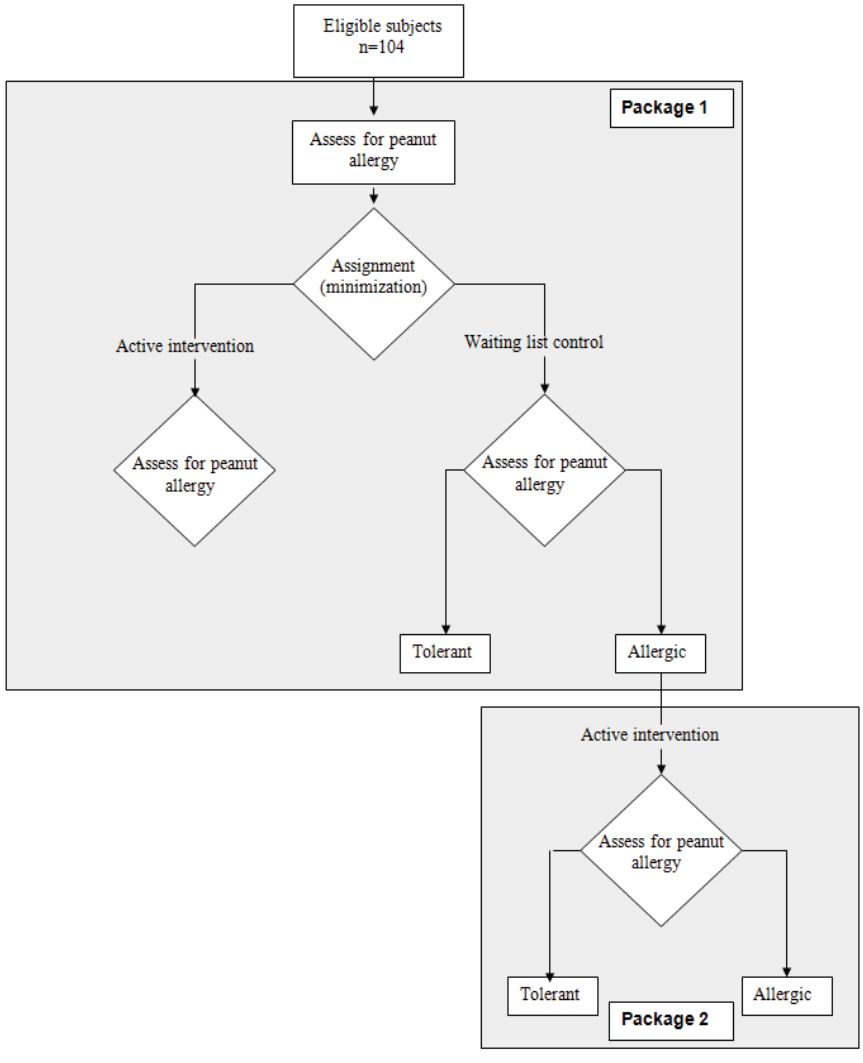
5 Study Methods

5.1 General Study Design and Plan

Single centre, randomized controlled trial of a novel active intervention (peanut oral immunotherapy) versus the status quo (peanut avoidance) in patients with peanut allergy. Two phase (package) design.

- Phase 1: Subjects randomly allocated (using minimisation methods – 80% chance of being assigned to imbalance minimising arm) to active intervention arm or waiting list arm. After 6 months all patients will be assessed for peanut allergy.
- Phase 2: Participants in waiting list arm still allergic to peanut at the end of phase 1 will be given the active intervention.

The figure below shows the study design.



5.2 Inclusion-Exclusion Criteria and General Study Population

5.2.1 Inclusion Criteria

1. Subjects aged between 7 and 15 years of age

2. Subjects with peanut allergy confirmed by a clinical history of a typical rapid onset immediate type hypersensitivity reaction to definite peanut ingestion.

3. Positive skin prick test to peanut (extract ALK-Abello, Hørsholm, Denmark) defined by wheal ≥3mm in the presence of a negative control and positive histamine control.

4. Positive double blind placebo controlled food challenge performed according to international consensus guidelines

5. Informed consent obtained from parent / guardian or participant, as appropriate.

5.2.2 Exclusion Criteria

1. Clinically significant chronic illness, except for eczema, rhinitis or asthma.

2. Suspected or diagnosed allergy to peanut protein in care provider or current household member.

3. Unwillingness or inability to comply with study requirements and procedures.

5.3 Randomisation and Blinding

Subjects were allocated using minimisation to avoid imbalance of confounding factors between groups, with a random element using a weighting probability of 0.8 – i.e. patients were allocated to the group which would provide optimal balance with 80% probability.

The minimising variables were age (7-<12, 12-15), sex, allergy severity (mild, moderate, severe, never eaten), asthma, serum peanut IgE (≤28kU/l, >28kU/l), challenge threshold (>5mg, 50-200mg, >200mg) and other current food allergies.

Patients are not blinded to their randomised groups. However the primary outcome will be assessed using a double blind placebo controlled food challenge (DBPCFC). On the challenge neither the investigator, nurse not the participant will know whether the participants dose is peanut or placebo.

5.4 Study Variables

The visit schedule is shown below:

		Phase 1										*Phase 2*									
Week	0	2	4	6	8	10	12	14	16	18	24	*26**	*28**	*30**	*32**	*34**	*36**	*38**	*40**	*42**	*48**
Active immunotherapy visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	–	–	–	–	–	–	–	–	–	–
Waiting list visit*	**(Baseline)**	–	–	–	–	–	–	–	–	–	**2**	***3****	***4****	***5****	***6****	***7****	***8****	***9****	***10****	***11****	***12****
Demographics	x																				
Other allergies	x																				
WAO score (historical)	x																				
DBPCFC (NOAEL, LOAEL, WAO score)	x										x										*x*
Peanut SPT	x										x										*x*
Other nut SPT	x										x										*x*
Peanut specific IgE	x										x										*x*
Total IgE	x										x										*x*
Basophil histamine release	x										x										*x*
Tryptase	x																				
QoL questionnaire	x										x										*x*
OIT updose*		x	x	x	x	x	x	x	x	x		*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	

**Only waiting list patients who are not desensitised to peanut after 24 weeks undergo immunotherapy from week 26 to 48*

5.4.1 Important Variable Definitions

- Peanut threshold (mg) – The no observed adverse effect level (NOAEL) – the highest cumulative dose of peanut that does not cause a reaction in a DBPCFC
- The lowest observed adverse effect level (LOAEL) is the lowest cumulative dose that causes a reaction (mg) in a DBPCFC
- Peanut IgE (kU/l 0 -1000) – Specific allergy antibody against peanut protein. Higher titres of IgE antibodies indicates higher probability of clinical allergy
- Basophil histamine release – Donor basophils stimulated in vitro by peanut extract at a range of concentrations. Basophil stimulation is detected by the mean fluorescent intensity of a cell surface marker (CD63). Area under the curve (AUC) of mean fluorescent intensity of CD63 (arbitrary units) and of the %CD63 positive cells against dose of peanut protein in milligrams.
- Tryptase (ng/ml) – a marker of background mast cell activation
- SPT diameter (mm 0-20) – A small amount of purified peanut is pricked on to the skin, and the diameter of the resulting wheal is recorded.

6 Sample Size

Based on Fisher's exact test with 90% power and 5% significance (two-sided) a sample size of 49 in each group is sufficient to detect proportions of participants with desensitisation to peanut of 64% and 30% in the intervention and control group respectively. Allowing for 5% drop out increases the sample size to 52 participants in each group and 104 subjects overall.

Based on the above we would expect 35 waiting list group patients to proceed to the active intervention in phase 2. To confirm efficacy of immunotherapy the success rate of all participants (intervention and phase 2 waiting list) should be within 10% of the success rate seen in intervention participants alone in phase 1.

7 General Considerations

7.1 Analysis Populations

7.1.1 Full Analysis Population

All subjects who were randomised and participated in at least one post-baseline assessment

7.1.2 Per-Protocol Population

The criteria for per-protocol analysis of the outcome of peanut challenge at the end of immunotherapy will consist of: desensitisation and continuation of immunotherapy up to the maintenance dose of 800mg protein.

7.1.3 Safety Population

All subjects who received any study treatment (including control) and are confirmed as providing complete follow-up regarding adverse event information.

7.2 Covariates and Subgroups

The covariates that are recorded at baseline are:

- Demographics (age, sex, weight, family history of allergy)
- Other allergic diseases (asthma, eczema, rhinitis)
- Sensitisation to tree nuts
- Severity of worst reaction before enrolment (WAO score)
- DBPCFC – WAO score, LOAEL, NOAEL
- Peanut SPT wheal diameter
- Other nut SPT wheal diameter
- Peanut specific IgE
- Total IgE
- Basophil AUC of CD63MFI against peanut protein concentration
- Basophil AUC of %CD63 positive cells against peanut protein concentration
- Tryptase
- QoL score

7.3 Missing Data

The frequency of missing data for all variables used in analysis will be summarised in a table.

To avoid using only complete case data for regression analysis when baseline covariates are missing, the 'missing indicator' method will be used. That is, we will include a binary indicator variable for the missingness of each covariate in regression models and we will 'fill in' all missing baseline covariate values with a fixed arbitrary number, say 0, to avoid software programs automatically omitting non complete cases from analysis.

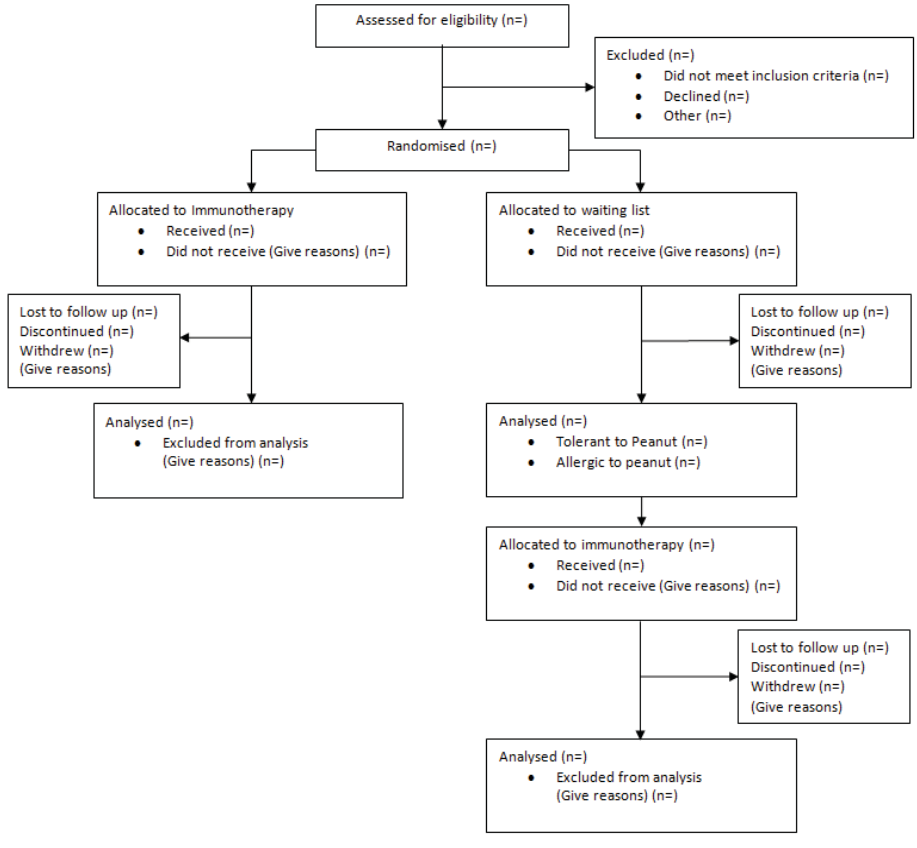
8 Summary of Study Data

Summaries of the study data will be presented as tables with separate columns for randomised group. All randomised patients will be included regardless of those who may not have completed the study. A table of baseline characteristics of patients will be included with a row for each covariate described in section 7.2.

Continuous variables will be summarised using means and standard deviations (or median and interquartile range if more appropriate) and categorical variables will be summarised using frequencies and percentages. The extent of missing data will be reported using annotations for the table and graphically using plots

8.1 Subject Disposition

Below is a blank example of a CONSORT diagram to include in the final report



8.2 Protocol Deviations

The summary statistics will be produced in accordance with section 8.

8.3 Demographic and Baseline Variables

The summary statistics will be produced in accordance with section 8.

8.4 Concurrent Illnesses and Medical Conditions

The summary statistics will be produced in accordance with section 8.

8.5 Prior and Concurrent Medications

The summary statistics will be produced in accordance with section 8.

8.6 Treatment Compliance

The summary statistics will be produced in accordance with section 8.

9 Efficacy Analyses

All statistical tests described in this section use a 2-sided 5% significance level. Non-parametric tests may be used instead of parametric tests if the assumptions are not appropriate. Sensible transformations of data may be used to allow the data to fulfil assumptions.

Plots (e.g. histograms, residuals) will be used to assess the assumptions and fits of regression models.

Tests will be accompanied with a measure of effect size (e.g. difference, odds ratio etc.) and a 95% confidence interval.

All analyses will be performed on an intention to treat (ITT) basis.

9.1 Primary Efficacy Analysis

Fisher's exact test will be used to compare the incidence with desensitisation to peanut after 6 months between the intervention and waiting list group.

Multiple logistic regression will be used to adjust the odds of desensitisation for baseline characteristics described in section 7.2.

9.2 Secondary Efficacy Analyses

- The incidence of desensitisation to peanut in the waiting list group after receiving the active intervention (end of phase 2) will be reported and compared with the incidence of desensitisation to peanut in the intervention group (end of phase 1) using Fishers exact test.

- Incidence of response to treatment (i.e. desensitisation to the maintenance immunotherapy dose of 800mg) will be compared between the intervention and waiting list groups using Fishers exact test. Multiple logistic regression will be used to adjust the odds ratio of response to treatment for baseline characteristics described in section 7.2.
- The absolute and fold change in threshold will be compared within and between groups using paired and independent sample t-tests respectively. This may be extended to a linear regression adjusting for baseline covariates (section 7.2)
- QoL scores at the end of phase 1 will be compared between groups using a Mann Whitney U test.
- Change in QoL scores before and after intervention in both groups (Intervention: Phase1 – baseline; Waiting list: Phase 2- Phase 1) will be investigated within groups using a Wilcoxon sign rank test and then between groups using a Mann –Whitney U test.
- Change in peanut IgE from baseline to end of phase 1 will be compared between groups using a Mann-Whitney U test.
- Change in AUC (CD63MFI and %CD63 positive cells) of Basophil histamine release from baseline to end of phase 1 will be compared between groups using a Mann-Whitney U test
- Change in SPT results (peanut and other nuts) from baseline to end of phase 1 will be compared between groups using a Mann- Whitney U test
- Change in WAO score from baseline to end of phase 1 will be compared between groups using a Mann – Whitney U test

10 Safety Analyses

10.1 Extent of Exposure

The summary statistics will be produced in accordance with section 8.

10.2 Adverse Events

A table summarising the incidence of the following adverse events per peanut dose will be produced: mouth itching, abdominal pain, nausea, vomiting, diarrhoea, urticaria, angioedema, erythema, rhinitis, wheezing, laryngeal oedema, cardiovascular collapse, fainting, admission to intensive care unit (ITU), serious

adverse reaction, suspected unexpected serious adverse event (SUSAR), use of nebulised beta 2 agonist and use of intramuscular adrenaline.

10.3 Deaths, Serious Adverse Events and other Significant Adverse Events

The summary statistics will be produced in accordance with section 8.

11 Figures

The figures that will be produced include, but are not limited to:

Box plots:

- Peanut threshold at baseline and end of phase 1 (and phase 2 for waiting list group) by group
- Serum peanut specific IgE (log) at baseline and end of phase 1(and phase 2 for waiting list group) by group
- SPT at baseline and end of phase 1 (and phase 2 for waiting list group) by group

Plot of basophil histamine release CD63MFI and %CD63 positive cells against peanut protein concentration (baseline and phase 1 (and phase 2 for waiting list group)) by group

Plot of probability of desensitisation to peanut according to logistic regression model with a continuous independent covariate on the x-axis (possibly baseline peanut IgE, threshold or age), separate lines for treatment groups and appropriate constants chosen for all other covariates.

Plot of probability of response to treatment to peanut according to logistic regression model with a continuous independent covariate on the x-axis (possibly baseline peanut IgE, threshold, or age), separate lines for treatment groups and appropriate constants chosen for all other covariates.

12 Reporting Conventions

P-values $\geq$0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

13 Listing of Tables and Figures

A summary of the tables and figures to be included in the final report are given in the tables in this section

13.1Tables

Table Title	No.	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis
Summary of patient characteristics	1.1	Full analysis	Age	Baseline	Treatment	n, mean, SD	NA
			Sex			n, %	
			Weight			n, mean, SD	
			Asthma			n, %	
			Eczema			n, %	
			Rhinitis			n, %	
			Other food allergy			n, %	
			Tryptase			n, mean, SD	
			WAO score (historical)			n, median, range	
			Sensitisation to tree nuts			n, %	
			QoL score			n, median, range	
			Peanut specific IgE			n, median, range	
			Total IgE			n, median, range	
			Peanut SPT wheal diameter			n, mean, SD	
			Other nut SPT wheal diameter			n, mean, SD	
			Family history of peanut allergy			n, %	
			DBPCFC total peanut protein consumed			n, median, range	
			DBPCFC peanut threshold (NOAEL)			n, mean, SD	
			DBPCFC WAO score			n, median, range	
			DBPCFC LOAEL			n, mean, SD	

			Basophil AUC of CD63MFI against peanut protein concentration			n, mean , SD	
			Basophil AUC of %CD63 positive cells against peanut protein concentration			n, mean, SD	
Desensitisation to peanut after 6 months	2.1	Full analysis	Allergy desensitised or undesensitised	6 months	Treatment	n, %, odds ratio, 95% CI	Fishers exact test
Logistic regression – adjusted odds of desensitised peanut allergy	2.2	Full analysis	Allergy desensitised or undesensitised	6 months	Treatment Age Sex Asthma Eczema Rhinitis Weight Sensitisation to tree nuts Peanut specific IgE Peanut SPT wheal diameter Basophil AUC of CD63MFI against peanut protein concentration QoL score Family history of allergy Baseline NOAEL	odds ratios, 95% CI	Logistic regression
Comparison of change in threshold between treatment groups	2.3	Full analysis	Threshold	Baseline and 6 months	Treatment	mean, SD, absolute and fold difference, 95% CI	t-test

Comparison of change in quality of life score between treatment groups	2.4	Full analysis	Quality of life score	Baseline and 6 months	Treatment	median, range, difference, 95% CI	Mann-Whitney U test
Comparison of change in quality of life score before and after intervention within and between treatment groups	2.5	Full analysis	Quality of life score	Baseline and 6 months (immunotherapy) 6 and 12 months (Waiting list)	Treatment	median, range, difference, 95% CI	Wilcoxon sign rank test Mann-Whitney U test
Comparison of change in peanut immunological outcomes between treatment groups	2.6	Full analysis	Peanut IgE Total IgE Peanut SPT Other nut SPT Basophil histamine release CD63MFI (AUC) Basophil histamine release %CD63 positive cells (AUC)	Baseline and 6 months	Treatment	median, range, difference, 95% CI	Mann-Whitney U tests
Comparison of change in WAO score between treatment groups	2.7	Full analysis	WAO score	Baseline and 6 months	Treatment	median, range, difference, 95% CI	Mann-Whitney U tests
Predictors of treatment response	2.8	All patients who underwent immunotherapy	Response to immunotherapy	6 months (or 12 months for Waiting list group)	Treatment Age Sex Asthma Eczema Rhinitis Weight	odds ratios, 95% CI	Logistic regression

					Sensitisation to tree nuts Peanut specific IgE Peanut SPT wheal diameter Basophil AUC of CD63MFI against peanut protein concentration QoL score Family history of allergy peanut Baseline NOAEL		
Adverse events per dose	2.9	Safety population	Mouth itching Abdominal pain Nausea Vomiting Diarrhoea Urticaria Angioedema Erythema Rhinitis Wheezing Laryngeal oedema Cardiovascular collapse Fainting Admission to ITU Serious adverse reaction SUSAR	End of study	Treatment	n, %	NA

	Use of nebulised beta 2 agonist	
	Use of intramuscular adrenaline	

13.2 Figures

Title	No.	Population	Type of graph	Horizontal Variables	Vertical Variables	Groupings
CONSORT diagram	1.1		Flow chart			
Peanut Threshold	2.1	Full analysis	Box plot	Peanut threshold (NOAEL) (mg)	NA	Treatment and time (Baseline, end of phase 1 and end of phase 2)
Peanut IgE	2.2	Full analysis	Box plot	Peanut IgE (kU/L); log	NA	Treatment and time (Baseline, end of phase 1 and end of phase 2)
Skin Prick Test	2.3	Full analysis	Box plot	Peanut SPT diameter (mm)	NA	Treatment and time (Baseline end of phase 1 and end of phase 2)
Basophil histamine release	2.4	Full analysis	Line	Peanut concentration	mean fluorescent intensity of CD63	Treatment and time (Baseline, end of phase 1 and end of phase 2)
Basophil histamine release	2.5	Full analysis	Line	Peanut concentration	%CD63 positive cells	Treatment and time (Baseline, end of phase 1 and end of phase 2)
Probability of desensitisation	2.6	Full analysis	Line	Baseline NOAEL	Probability	Treatment
Probability of treatment response	2.7	Full analysis	Line	Baseline NOAEL	Probability	Treatment