

Worked examples of human clinical studies, to illustrate activity which falls outside the scope of the Clinical Trials Directive, in the context of microbicides and vaccines to prevent HIV infection.

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The "EUROPRISE" Network of Excellence is a European Commission funded programme to advance the development of microbicides and vaccines against HIV. One task is to assist researchers in this field by providing advice and information on the impact of the Clinical Trials Directive on studies involving human subjects and pharmaceutical agents, which are not intended as trials of novel vaccines or microbicides.

The following examples are based on recent guidance issued by MHRA on what constitutes a "Clinical Trial" within the scope of the Clinical Trials Directive, and what is an "Investigational Medicinal Product (IMP)" and what is a "Non-Investigational Medicinal Product (NIMP)". They present applications which will be meaningful to clinical researchers in the field of HIV, vaccines and microbicides.

These examples have been developed in consultation with the Clinical Trials Unit of the UK MHRA.

Readers are directed to the table of abbreviations and definitions at the end of this document for a description of the terms "clinical trial", "investigational medicinal product", etc.

These examples must be read as hypothetical guides, and investigators should not rely on their studies being classified the same way by their national or local authorities, even if very similar. The science presented here is also merely illustrative and has been made-up.

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Key Concepts

Challenge agents *versus* investigational agents

A key concept in these examples is the idea of a "challenge agent"¹. This is a tool that we use to reveal or induce a *physiological* process, so that we can then study the *physiological process*. Once the physiological process is revealed, the challenge agent conceptually fades into the background, it has done its job. It is a tool in the experiment, and not the subject of the experiment. In these examples radiology contrast gel is a tool to literally reveal a physiological process, and vaccines are tools to induce antibodies and cells that can then be studied in an abstract way.

In contrast, an "investigational agent"² is the subject and object of the experiment, and we study the effects it has on physiology to learn more about it - the agent itself.

What do you want to study?

When an active agent interacts with human physiology, there will be a spectrum of events that can be observed. At one end will be events very closely associated with, and highly specific to, the active agent itself. Measuring these events will tell you something specific to the agent. In contrast there will be events that are more remote, unrelated and abstract from the particular agent, that can be studied in isolation - almost without ever knowing which agent provoked them.

A key question you must ask is "am I studying the agent, or the physiology?" If you are studying the *agent*, broadly speaking it is likely to be a clinical *trial*.

If you really care very little about the agent, and your interest is focused on the *physiology*, then it may be a challenge agent used in a "NIMP" *study*, and not a trial.

One must determine in advance which end of the spectrum one is placing the hypotheses to be tested: looking at the agent, or looking at physiology?

Once this is clearly determined it must be very clearly communicated in the protocol title, objectives and aims. In this way a regulator or reviewer can see at once if it is a study or a trial.

*The way you word the title, phrase your objectives, and describe the aims will be crucial in whether it is genuinely perceived to be a Clinical Trial or a NIMP study. **You should take great care over this**, as certain trigger phrases and terms may convey significance to a regulator or reviewer that you did not intend. For example if you refer to "safety" or "efficacy" it is likely to be seen as a trial.*

¹ See definition and references in Table of Abbreviations and Definitions

² See definition and references in Table of Abbreviations and Definitions

Concept 1: “Measuring the uterine peristaltic pump”

The worked examples below are all variations of an experiment to study the “uterine peristaltic pump”. For the purpose of these scenarios it is hypothesized that a peristaltic pumping action of the uterus can actively transport the contents of the vaginal cavity up into the uterus. This peristaltic pump is thought to be affected by hormones controlling the menstrual cycle, being stronger at certain times of the cycle. A mixture of a jelly with radiology grade gadolinium (an MRI contrast medium) is used to quantify the uptake of vaginal contents into the uterus. By measuring the changes in MRI intensity of uterine and vaginal lumens over a period of time after the jelly mixture is introduced into the vagina, the rate of uptake can be quantified.

| 1. Healthy Genital Tract Physiology | | |
|--|---|-------------------------|
| Trial Design | A study of vagino-uterine transport in healthy volunteers | |
| Purpose of trial | To investigate intra-and inter-subject variability of vagino-uterine transport during the menstrual cycle | |
| Products Administered | A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> • 5 mL “over the counter” lubricant jelly for topical intravaginal instillation • 0.2 mL radiology-grade gadolinium for topical intravaginal instillation | |
| Key Parameters | Uptake of vaginal luminal contents in the same subject at different times in the menstrual cycle | |
| Product Classification | Lubricant jelly | NIMP |
| | Gadolinium | NIMP |
| Rationale for Clinical Trials Directive status | <p>This study is investigating basic female reproductive tract physiology. Data can be extrapolated to inform basic models of fertility (sperm uptake), infection (pathogen uptake) or potentially medicinal product uptake (e.g. vaccines or microbicides).</p> <p>Lubricant Jelly provides a bulking agent to trap the gadolinium - a registered MRI contrast agent being used to reveal gel uptake. Neither are presented as a medicinal or diagnostic product.</p> <p>Since no investigational medicinal products are being administered, this is not a clinical trial within the scope of the Clinical Trials Directive</p> | Non-CTIMP |
| GMP Directive status | The lubricant jelly & gadolinium mixture is the medicinal product used but will be a NIMP and an MA(IMP) is not required | No MA(IMP) ³ |
| Rationale for required GxP | As this is not a clinical trial, no GxP are required | No GxP ⁴ |

³ While an MA(IMP) is not needed, these are still medicinal products for human use and must be handled and stored appropriately, according to local guidelines and regulations.

⁴ Although full GxP may not be required, investigators must abide by ethical and other regulatory or governance requirements, as with all human experimentation.

| 2. Pathophysiology Of Genital Tract Disease | | |
|--|---|------------|
| Trial Design | A single-blind study of vagino-uterine transport in female infertility patients and healthy volunteers | |
| Purpose of trial | To investigate between-group difference in uterine uptake of vaginal contents | |
| Products Administered | A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> • 5 mL “over the counter” lubricant jelly for topical intravaginal instillation • 0.2 mL radiology-grade gadolinium for topical intravaginal instillation | |
| Key Parameters | <ul style="list-style-type: none"> • Uptake of vaginal luminal contents in subjects at the same time in the menstrual cycle • Radiologists are blinded to the fertility status of the subjects. • The level of uptake is compared between “normal” and “abnormal” population | |
| Product Classification | Lubricant jelly | NIMP |
| | Gadolinium | NIMP |
| Rationale for Clinical Trials Directive status | <p>This study is investigating healthy and abnormal female reproductive tract physiology. Data can be extrapolated to models of fertility (sperm uptake).</p> <p>Lubricant Jelly provides a bulking agent to trap the gadolinium - a registered MRI contrast agent being used to reveal gel uptake. Neither are presented as a medicinal or diagnostic product.</p> <p>Even though the study is explicitly measuring differences between groups, as no investigational medicinal products are being administered, this is not a clinical trial within the scope of the Clinical Trials Directive</p> | Non-CTIMP |
| GMP Directive status | The lubricant jelly & gadolinium mixture is the medicinal product used but will be a NIMP and an MA(IMP) is not required | No MA(IMP) |
| Rationale for required GxP | As this is not a clinical trial, no GxP are required | No GxP |

| 3. Healthy Genital Tract Physiology including subjects taking a Pharmacological Agent from a Group | | |
|--|--|------------|
| Trial Design | A study of vagino-uterine transport in healthy volunteers, not excluding women taking any Oral Contraceptive Pill (OCP) from a defined group of agents | |
| Purpose of trial | To investigate intra-and inter-subject variability of vagino-uterine transport in healthy subjects not excluding women already taking any combined monophasic OCP | |
| Products Administered | 1. A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> • 5 mL “over the counter” lubricant jelly for topical intravaginal instillation • 0.2 mL radiology-grade gadolinium for topical intravaginal instillation 2. Any licensed combined “monophasic” OCP | |
| Key Parameters | Uptake of vaginal luminal contents in the same subject at different times in the menstrual cycle | |
| Product Classification | Lubricant jelly | NIMP |
| | Gadolinium | NIMP |
| | Monophasic combined OCP | NIMP |
| Rationale for Clinical Trials Directive status | <p>The study is being undertaken by an academic group without any links to a manufacturer of an OCP.</p> <p>The study is investigating basic female reproductive tract physiology in a representative population. In contrast to example 1, women will be allowed to participate if they are already taking a monophasic OCP. Subsequent data analysis will include OCP use, to determine if the hormones of the OCP may influence the physiology. However, the key point is that women will be included whether or not they are on the OCP - it is not the focus of the study, just one of several factors than can subsequently be evaluated such as age, etc. In effect the investigators are just collecting a medical history of monophasic OCP use, and excluding women on other forms of contraception.</p> <p>To partially standardize the study, participants are allowed to participate if they are already stable on a monophasic OCP which they are using as a contraceptive. However no specific product is specified in the protocol, any monophasic combined OCP is suitable, and no changes to pre-study medication is made, the OCP being taken for its licensed indication as a contraceptive, and the drug history recorded.</p> <p>The generic OCP in this example is not an IMP and the study not a clinical trial.</p> <p>Lubricant Jelly provides a bulking agent to trap the gadolinium - a registered MRI contrast agent being used to reveal gel uptake. Neither are presented as a medicinal or diagnostic product.</p> | Non-CTIMP |
| Rationale for GMP Directive status | The generic OCP is a NIMP | No MA(IMP) |
| | As jelly & gadolinium mixture is the product used but will be a NIMP an MA(IMP) is not required | No MA(IMP) |
| Rationale for required GxP | As this is not a clinical trial, no GxP are required | No GxP |

| 4. Effect of a Pharmacological Agent from a Group | | |
|---|---|---------------------|
| Trial Design | A study of vagino-uterine transport in healthy volunteers taking any Oral Contraceptive Pill (OCP) from a defined group of agents | |
| Purpose of trial | To investigate intra-and inter-subject variability of vagino-uterine transport in subjects already taking any combined monophasic OCP and those not on any OCP | |
| Products Administered | 1. A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> 5 mL "over the counter" lubricant jelly for topical intravaginal instillation 0.2 mL radiology-grade gadolinium for topical intravaginal instillation 2. Any licensed combined "monophasic" OCP | |
| Key Parameters | Uptake of vaginal luminal contents in the same subject at different times in the menstrual cycle | |
| Product Classification | Lubricant jelly | NIMP |
| | Gadolinium | NIMP |
| | Monophasic combined OCP | IMP |
| Rationale for Clinical Trials Directive status | <p>The study is being undertaken by an academic group without any links to a manufacturer of an OCP.</p> <p>The protocol states that "the study is <i>investigating the effects</i> of a group of pharmacological agents – monophasic OCPs - on basic female reproductive tract physiology". Data can be used to inform OCP interactions with basic models of fertility (sperm uptake), infection (pathogen uptake) or potentially medicinal product uptake (e.g. vaccines or microbicides).</p> <p>To partially standardize the study, participants in the OCP group are selected on the basis that they are already stable on a monophasic OCP which they are using as a contraceptive. However no specific product is specified in the protocol, any monophasic combined OCP is suitable, and no changes to pre-study medication is made, the OCP being taken for its licensed indication as a contraceptive.</p> <p>However, unlike the previous example, this protocol explicitly separates subjects into groups according to OCP use, and is designed to <i>study the effects</i> of the OCPs as its main focus. Therefore, the generic OCP in this example should be considered an IMP and the study a Phase IV trial. Since it is not a specific OCP but a group, then in section D2 of the CTA form the reference can be 'generic'.</p> <p>The fact that subjects are already on the OCP, and that it may be prescribed by their regular physician, does not avoid this being a CTIMP.</p> <p>Lubricant Jelly provides a bulking agent to trap the gadolinium - a registered MRI contrast agent being used to reveal gel uptake. Neither are presented as a medicinal or diagnostic product.</p> | CTIMP |
| Rationale for GMP Directive status | The generic OCP is an IMP in a clinical trial it must be manufactured or imported by the holder of an MAIMP (or equivalent) | MA(IMP) |
| | As jelly & gadolinium mixture is the product used but will be a NIMP an MA(IMP) is not required | No MA(IMP) |
| Rationale for required GxP | As this is a clinical trial, GxP are required SUSAR reporting required ⁵ | GCP, GPvP, GLP/GCLP |

⁵ SUSAR reporting is required only for the IMPs and not the NIMPs. However, AEs should be recorded for both NIMPs and IMPs.

| 5. Effect of a Specified Pharmacological Agent On Healthy Physiology: no change in treatment | | |
|--|---|---------------------|
| Trial Design | A study of vagino-uterine transport in healthy volunteers taking a specified OCP | |
| Purpose of trial | To investigate intra-and inter-subject variability of vagino-uterine transport in subjects already taking a specified OCP and those not on any OCP | |
| Products Administered | 1. A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> • 5 mL “over the counter” lubricant jelly for topical intravaginal instillation • 0.2 mL radiology-grade gadolinium for topical intravaginal instillation 2. “Mopylon” a licensed combined “monophasic” low-dose oral contraceptive | |
| Key Parameters | Uptake of vaginal luminal contents in the same subject at different times in the menstrual cycle | |
| Product Classification | Lubricant jelly | NIMP |
| | Gadolinium | NIMP |
| | ‘Mopylon’ OCP | IMP |
| Rationale for Clinical Trials Directive status | <p>This study is investigating the effects of OCPs on basic female reproductive tract physiology. Data can be used to inform OCP interactions with basic models of fertility (sperm uptake), infection (pathogen uptake) or potentially medicinal product uptake (e.g. vaccines or microbicides).</p> <p>The study is being undertaken by an academic group without any links to a manufacturer of an OCP.</p> <p>To maximise homogeneity of data, participants in the OCP group are selected on the basis that they are already taking a specific OCP “Mopylon” which they are using as a contraceptive. “Mopylon” was chosen to simplify recruitment as it is the most commonly prescribed OCP in the subject population. No changes to medication are made, Mopylon is used for its licensed indication as an OCP.</p> <p>‘Mopylon’ OCP in this example should be considered an IMP and the study a Phase IV trial.</p> <p>The fact that subjects are already on the OCP, and that it may be prescribed by their regular physician, does not avoid this being a CTIMP.</p> <p>Lubricant Jelly provides a bulking agent to trap the gadolinium - a registered MRI contrast agent being used to reveal gel uptake. Neither are presented as a medicinal or diagnostic product.</p> | CTIMP |
| Rationale for GMP Directive status | As ‘Mopylon’ is an IMP in a clinical trial it must be manufactured or imported by the holder of an MAIMP (or equivalent) | MA(IMP) |
| | As jelly & gadolinium mixture is the product used but will be a NIMP an MA(IMP) is not required | No MA(IMP) |
| Rationale for required GxP | As this is a clinical trial, GxP are required SUSAR reporting required | GCP, GPvP, GLP/GCLP |

| 6. Effect Of a novel OCP On Healthy Physiology | | |
|--|---|---------------------|
| Trial Design | A study of vagino-uterine transport in healthy volunteers taking a novel Oral Contraceptive Pill | |
| Purpose of trial | To investigate intra-and inter-subject variability of vagino-uterine transport in subjects taking a novel oral contraceptive and those with normal menstrual cycles not on any OCP | |
| Products Administered | 1. A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> • 5 mL “over the counter” lubricant jelly for topical intravaginal instillation • 0.2 mL radiology-grade gadolinium for topical intravaginal instillation 2. “NewPill” a novel combined “monophasic” low-dose oral contraceptive | |
| Key Parameters | Uptake of vaginal luminal contents in the same subject at different times in the menstrual cycle | |
| Product Classification | Lubricant jelly | NIMP |
| | Gadolinium | NIMP |
| | ‘NewPill OCP | IMP |
| Rationale for Clinical Trials Directive status | <p>This study is investigating the effects of the unlicensed OCP “NewPill” on basic female reproductive tract physiology as part of its safety evaluation. Data can be used to inform interactions with basic models of fertility (sperm uptake), infection (pathogen uptake) or potentially medicinal product uptake (e.g. vaccines or microbicides).</p> <p>“NewPill” is presented as an IMP in the context of uterine transport</p> <p>Lubricant Jelly provides a bulking agent to trap the gadolinium - a registered MRI contrast agent being used to reveal gel uptake. Neither are presented as a medicinal or diagnostic product.</p> <p>As an investigational medicinal product is being administered, this is a Phase 1 or 2 clinical trial within the scope of the Clinical Trials Directive</p> | CTIMP |
| Rationale for GMP Directive status | NewPill: As this is an IMP in a clinical trial it must be manufactured or imported by the holder of an MA(IMP) (or equivalent) | MA(IMP) |
| | Gadolinium: As a NIMP it need not be made to GMP | No MA(IMP) |
| | As jelly & gadolinium mixture is the product used but will be a NIMP an MA(IMP) is not required | No MA(IMP) |
| Rationale for required GxP | As this is a clinical trial, full GxP are required SUSAR reporting required | GCP, GPvP, GLP/GCLP |

| 7. Evaluation of a Diagnostic Kit | | |
|--|--|---------------------|
| Trial Design | An open-label single-blind study of a diagnostic gel in female infertility patients and healthy volunteers | |
| Purpose of trial | To investigate inter-subject and between-group variability of uterine uptake of a diagnostic vaginal gel in healthy and infertile women, and positive/negative predictive value of uptake as an infertility test. | |
| Products Administered | <p>"InfertiGelDX": A mixture of the following components, presented in a single-use disposable syringe for topical intra-vaginal instillation:</p> <ul style="list-style-type: none"> • 5 mL "lubricant jelly" • 0.2 mL radiology-grade gadolinium <p>Although the intention is to have a pre-mixed final product, for this trial individual components are provided in bulk to the Pharmacy which must aliquot and mix the final product, place it into a single-use syringe and dispense it to the investigator.</p> | |
| Key Parameters | Uptake of vaginal luminal InfertiGelDX in subjects at the specified point in the menstrual cycle | |
| Product Classification | "InfertiGelDX" | IMP |
| Rationale for Clinical Trials Directive status | <p>This study is investigating a new diagnostic test kit. Radiologists, blinded to the fertility status of the subjects will quantify the level of uptake at a defined point in the menstrual cycle in "normal" and "diseased" subjects. The inter-subject variability of measured uptake will determine the reproducibility of the test, and the ability of measured uptake to identify infertile subjects will define specificity/sensitivity.</p> <p>The product is presented as a <i>diagnostic kit</i>. The efficacy of investigational medicinal products is therefore being tested. This is a clinical trial within the scope of the Clinical Trials Directive</p> | CTIMP |
| Rationale for GMP Directive status | As this is a clinical trial the individual components must be made to GMP, and the final mixing of components in the Pharmacy is a manufacturing process. | MA(IMP) |
| Rationale for required GxP | As this is a clinical trial, full GxP are required SUSAR reporting required | GCP, GPvP, GLP/GLCP |
| Notes | <i>The safety could also be evaluated by recording reactions and adverse events</i> | |

This following examples introduce the concept of "mixing" and "manufacture" in a CTIMP. The fact that the mixing in this example occurred at the bedside is irrelevant. "Reconstitution" typically means adding-back something removed during manufacture, such as a diluent to a lyophilised vaccine. "Mixing" refers to adding together two components that do not bind to each other, modify, or change each other - simply mix together. While an MA(IMP) is not required, staff will still have to be designated, trained, and follow SOPs and local organisational guidelines about mixing medicinal products. Mixing may appropriately take place inside or outside a Pharmacy, and involve Pharmacists or other appropriately trained and supervised staff. If the mixing process involves several agents, or agents that modify each other, or complex measurements and timings, it is likely to be "manufacture" and require an MA(IMP).

| 8. Evaluation of a Microbicide | | |
|--|---|---------------------|
| Trial Design | An open-label, uncontrolled, single-blind study of topical intra-vaginal administration of "X1" microbicide in healthy volunteers | |
| Purpose of trial | To investigate intra-and inter-subject variability of vagino-uterine transport of X1 during menstrual cycle. | |
| Products Administered | A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> • 5 mL "X1" an anti-viral gel for topical intravaginal instillation • 0.2 mL radiology-grade gadolinium for topical intravaginal instillation | |
| Key Parameters | Uptake of vaginal luminal X1 in the same subject at different times in the menstrual cycle | |
| Product Classification | X1 | IMP |
| | Gadolinium | NIMP |
| Rationale for Clinical Trials Directive status | <p>This study is investigating whether the uptake of a new anti-HIV microbicide gel into the uterus is affected by menstrual cycle hormones. As the gel must remain in the uterus to exert its anti-HIV effect (by binding specifically to cervico-vaginal epithelia) its efficacy may be reduced at certain times in the menstrual cycle by increased removal from the vagina into the uterus. Radiologists, blinded to the menstrual cycle stage of the subjects will quantify the level of uptake at a different points in the menstrual cycle. The inter-subject variability of measured uptake will determine the effect of menstrual hormones on retention of X1 in the vagina and hence its efficacy when used at that time.</p> <p>X1 is presented as a medicinal product, the efficacy of which may be susceptible to uterine extraction. The efficacy of investigational medicinal products is therefore being tested. This is a clinical trial within the scope of the Clinical Trials Directive.</p> <p>The gadolinium - a registered MRI contrast agent – is being used to reveal X1 uptake, and although presented as a medicinal product is a NIMP in this trial</p> | CTIMP |
| Rationale for GMP Directive status | X1: As this is an IMP in a clinical trial it must be manufactured or imported by the holder of an MA(IMP) (or equivalent) | MA(IMP) |
| | Gadolinium: As a NIMP it need not be made to GMP | Non-GMP |
| | The mixing of an IMP and NIMP would not be considered manufacture and would not require an MA(IMP). However local regulations regarding handling medicinal agents will need to be followed. | No MA(IMP) |
| Rationale for required GxP | As this is a clinical trial, full GxP are required SUSAR reporting required | GCP, GPvP, GLP/GCLP |
| Notes | <i>The safety of X1 would probably have been tested already in a Phase 1 trial of X1 alone</i> | |

| 9. Evaluation of a Vaccine Delivery System | | |
|--|--|----------------------|
| Trial Design | An open-label, uncontrolled, single-blind study of topical intra-vaginal administration of antigen delivery system "UtoVax" in healthy volunteers | |
| Purpose of trial | To investigate intra-and inter-subject variability of vagino-uterine transport of UtoVax during menstrual cycle. | |
| Products Administered | A mixture, made at the bedside, of the following: <ul style="list-style-type: none"> • 5 mL "UtoVax" gel for topical intravaginal instillation • 0.2 mL radiology-grade gadolinium for topical intravaginal instillation | |
| Key Parameters | Uptake of vaginal luminal UtoVax in the same subject at different times in the menstrual cycle | |
| Product Classification | UtoVax | IMP |
| | Gadolinium | NIMP |
| Rationale for Clinical Trials Directive status | <p>UtoVax is a Rheologically Structured Vehicle – a foamy gel which traps vaccine antigens, and has a consistency which predisposes to uptake into the uterus from the vagina. By targeting antigens to the uterus it is thought to stimulate better mucosal immunity, but its efficacy may be reduced at certain times in the menstrual cycle if uptake is reduced. Radiologists, blinded to the menstrual cycle stage of the subjects will quantify the level of uptake at a different points in the menstrual cycle. The inter-subject variability of measured uptake will determine the effect of menstrual hormones on uptake of UtoVax into the uterus and hence its efficacy when used to immunize at that time.</p> <p>UtoVax is presented as a medicinal product, the efficacy of which may be susceptible to uterine extraction. The efficacy of an investigational medicinal product is therefore being tested. This is a clinical trial within the scope of the Clinical Trials Directive.</p> <p>The gadolinium - a registered MRI contrast agent – is being used to reveal X1 uptake, and although presented as a medicinal product is a NIMP in this trial.</p> | CTIMP |
| Rationale for GMP Directive status | UtoVax: As this is a clinical trial it must be made to GMP. | GMP |
| | Gadolinium: As a NIMP it need not be made to GMP | Non-GMP |
| | The mixing of an IMP and NIMP would not be considered manufacture and would not require an MA(IMP). However local regulations regarding handling medicinal agents will need to be followed. | No MA(IMP) |
| Rationale for required GxP | As this is a clinical trial, full GxP are required SUSAR reporting required | GCP, GPvP, GLP/GLCLP |
| Notes | <i>The safety of UtoVax would probably have been tested already in a Phase 1 trial of UtoVax alone.</i> | |

Concept 2: Using vaccines to induce immune responses

It has been speculated from animal models that sublingual lymph nodes are part of a "Common Mucosal Immune System" such that antigens presented to the sublingual nodes will induce a population of T and B cells that preferentially home to mucosal surfaces such as genital tract, gut and upper respiratory tract, rather than to the systemic immune system. In contrast injected antigens draining to systemic lymph nodes are thought not to induce mucosal immunity. By using flow cytometry to identify known patterns of surface markers on B and T cells associated with a "systemic" or "mucosal" homing preference, the cellular bias induced by sublingual antigen delivery can be compared with injected antigen draining to systemic lymph nodes. By comparing the levels of antibody in serum and various secretions, it can be determined whether indeed sublingual delivery of antigens stimulates a preferential "mucosal" bias in immune responses.

These examples highlight the difference between a "challenge agent" - a tool used to induce or reveal some physiological process to be studied, but which is itself *not* the focus of the study; and an "investigational agent" - which *is* the focus of a trial.

| 10. Healthy Physiology with an "irrelevant" vaccine given via two routes of administration | | |
|--|--|------------|
| Trial Design | A study of disseminated mucosal immune responses following sublingual antigen presentation | |
| Purpose of trial | To investigate the link between sublingual lymph node antigen stimulation with tetanus toxoid and immunity at distant mucosal surfaces in female healthy volunteers | |
| Products Administered | 1. Sublingual administration by instillation of drops of a licensed tetanus toxoid vaccine 2. Intramuscular administration of a licensed tetanus toxoid vaccine | |
| Key Parameters | 1. Anti-tetanus toxoid IgG and IgA antibody in cervico-vaginal secretions, saliva, and nasal wash 2. Serum anti-tetanus toxoid IgG and IgA antibody 3. Phenotype of circulating anti-toxoid T & B cells | |
| Product Classification | Sublingual tetanus toxoid vaccine challenge agent | NIMP |
| | Injected tetanus toxoid vaccine challenge agent | NIMP |
| Rationale for Clinical Trials Directive status | <p>This study is investigating basic mucosal immune system physiology, and is conducted by an academic group with funding from a non-commercial grant.</p> <p>A tetanus toxoid vaccine has been selected as a safe, well-defined model antigen preparation for which cellular and humoral immune assays are well established. As anti-tetanus immunity in mucosal secretions is not relevant for the efficacy against tetanus disease (which is transmitted through skin wounds) no new information on the efficacy of this vaccine is being collected. Serum antibody against tetanus toxoid is being measured, and is the known correlate of protection for this vaccine, but is not the focus of this study. The antibodies against tetanus toxoid are just tools to allow us to track immune responses induced at two different sites. The investigators have no interest in the vaccine, their focus is on the physiology. Even though a direct comparison of sublingual and parenteral delivery is made, this is an abstract concept not related to the specific vaccine.</p> <p>Tetanus antigens in the vaccine are being used as challenge agents for their known ability to induce an immune response, and the response is then being studied as a physiological event.</p> <p>The vaccine in this example is considered a challenge agent and not an IMP, and the study is not a CTIMP.</p> | Non-CTIMP |
| Rationale for GMP Directive status | Sublingual Tetanus toxoid vaccine: licensed preparation used as challenge agent | No MA(IMP) |
| | Injected Tetanus toxoid vaccine: licensed preparation used as challenge agent | No MA(IMP) |
| Rationale for required GxP | As this is not a clinical trial, no GxP are required | No GxP |

| 11. Healthy Physiology with a "relevant" vaccine | | |
|--|--|------------|
| Trial Design | A study of disseminated mucosal immune responses following sublingual antigen presentation | |
| Purpose of trial | To investigate the link between sublingual lymph node antigen stimulation with HPV glycoprotein and immunity at distant mucosal surfaces in healthy female volunteers | |
| Products Administered | 1. Sublingual administration by instillation of drops of a licensed human papilloma virus (HPV) vaccine 2. Intramuscular administration of a licensed HPV vaccine | |
| Key Parameters | 1. Anti-HPV glycoprotein IgG and IgA antibody in cervico-vaginal secretions, saliva, and nasal wash 2. Serum anti-HPV glycoprotein IgG and IgA antibody 3. Cellular phenotype of circulating anti-HPV glycoprotein T & B cells | |
| Product Classification | Sublingual HPV glycoprotein vaccine challenge agent | NIMP |
| | Injected HPV glycoprotein vaccine challenge agent | NIMP |
| Rationale for Clinical Trials Directive status | <p>This study is the same as example 11 except for the choice of vaccine. It has the same rationale - the vaccine antigens are being used as challenge agents to induce antibodies and T cells that can be used to track immune responses. The investigators have no interest in the vaccine, their focus is on the physiology.</p> <p>A licensed HPV glycoprotein vaccine has been selected as one to which the target population be immunologically naïve, and the glycoprotein would induce a primary and not a recall response.</p> <p>HPV is a genital infection, and anti-HPV immunity is a more relevant model of mucosal immunity than using tetanus toxoid. <i>The HPV vaccine is therefore a more relevant challenge agent for this model.</i></p> <p>In addition it was thought more ethical to use a vaccine that <i>may</i> confer some mucosal as well as systemic immunity to volunteers. Although, anti-HPV immunity in mucosal secretions is possibly relevant for efficacy against HPV, any new information on "efficacy" of this vaccine is only incidental, and is not relevant, or the focus of this study.</p> <p>The vaccine in this example is considered a relevant challenge agent for a study of responses to a neoantigen and genital tract immunity, and not an IMP, and the study is not a CTIMP.</p> | Non-CTIMP |
| Rationale for GMP Directive status | HPV glycoprotein vaccine: licensed preparation used as challenge agent. | No MA(IMP) |
| Rationale for required GxP | As this is not a clinical trial, no GxP are required | No MA(IMP) |

| 12. Evaluation of a novel sublingual vaccine | | |
|--|---|-----------------------------|
| Trial Design | An open-label, uncontrolled, single-blind study of genital mucosal immune responses following sublingual versus intramuscular immunisation | |
| Purpose of trial | To investigate intra-and inter-subject variability in the efficacy of sublingual or intramuscular immunisation with HPV glycoprotein to induce systemic and genital mucosal immunity in healthy female volunteers | |
| Products Administered | Group 1. Sublingual administration on 3 occasions by instillation of drops of a human papilloma virus (HPV) vaccine in single use, pre-filled applicators not requiring reconstitution Group 2. Intramuscular administration on 3 occasions of a licensed injected HPV vaccine | |
| Key Parameters | 1. Anti-HPV glycoprotein IgG and IgA antibody in cervico-vaginal secretions 2. Serum anti-HPV glycoprotein IgG and IgA antibody | |
| Product Classification | Sublingual HPV glycoprotein vaccine | IMP |
| | Injected HPV glycoprotein vaccine. As new information on the efficacy of this vaccine are being collected (mucosal immunity) this is an IMP. | IMP |
| Rationale for Clinical Trials Directive status | <p>This study is being conducted by the manufacturer of a licensed HPV vaccine to determine whether sublingual delivery is better at inducing genital tract immunity than intramuscular injection.</p> <p>It is proposed that a combination of mucosal and serum antibody against HPV glycoprotein may protect more effectively against HPV genital infection. This is a Phase 2a trial to determine whether sublingual delivery may offer an advantage over intramuscular injection in induction of mixed mucosal-systemic immunity.</p> <p>The study design is clearly focussed on the vaccines, and a physiological response is measured specifically to determine the efficacy of this new vaccine delivery, and not as an abstract model.</p> <p>As sublingual delivery of HPV vaccine is being clearly presented as a investigational medicinal product, this is a clinical trial within the scope of the Clinical Trials Directive.</p> | CTIMP |
| Rationale for GMP Directive status | HPV glycoprotein for sublingual delivery As this is a clinical trial it must be made to GMP. | MA(IMP) |
| | HPV glycoprotein for intramuscular delivery. As this is a clinical trial it must be made to GMP. As this is a licensed preparation an abbreviated dossier may be submitted and an MA(IMP) may not be required. | +/- MA(IMP) |
| | As the vaccines are presented in ready to use, single use applicators/syringes no manufacture is required at the clinical site | No MA(IMP) at clinical site |
| Rationale for required GxP | As this is a clinical trial, full GxP are required SUSAR reporting required | GCP, GvP, GLP/GCLP |

The manufacture of IMPs requires an MA(IMP), and this may include the final assembly of a product at a clinical site (typically in the Pharmacy) from bulk GMP-quality reagents supplied by the trial Sponsor. For example, in a dose-ranging study the Pharmacy may dilute a bulk vaccine antigen with buffer, and then label and dispense vials or syringes for subjects in each dose level group. Similarly a Pharmacy may mix a vaccine antigen with an adjuvant or buffer and dispense labeled units to be used in blinded trials where a vaccine with or without an adjuvant is compared, or there is a placebo group. In addition to Pharmacy time and costs, the pre-preparation of IMPs requires stability data to confirm the IMP will still be active after the intended period of storage in its final formulation.

However in open-label trials, or observer blinded trials, the clinical study staff may be fully aware of the vaccine combination they are administering, and so there may be no need to pre-prepare units for specific subjects or groups. In this case simple "bedside mixing" can be used to reduce costs and simplify the study design. Extended stability data on the mixture need not be gathered as it is used at once. A complex mixing process may well fall under "manufacture" and require an MA(IMP), but the simple bedside mixing of two components by well trained clinical staff may not.

The following is cited as an example where simple "bedside mixing" is used, for which the clinical study site does not need an MA(IMP) to "manufacture" the final vaccine preparation to GMP. All the bulk reagents will have been manufactured or imported by the holder of an MA(IMP).

| 1. Use of "bedside mixing" in the evaluation of a novel adjuvanted sublingual vaccine | | |
|---|--|---------------------|
| Trial Design | <p>An open-label, uncontrolled, single-blind study of genital mucosal immune responses following sublingual immunisation using a vaccine antigen - adjuvant mixture.</p> <p>The vaccine antigen and adjuvant come in separate vials (could be single-dose or multi-dose), as a solution in physiological buffer. To achieve the final dose of adjuvant the clinical study staff remove a fixed volume of adjuvant solution from one vial and add it to the vaccine antigen solution. The vaccine antigen solution vial is then used immediately for the sublingual immunization by removing and administering a fixed volume of the antigen-adjuvant mixture. Any excess is discarded or used within one working day.</p> <p>A vial of plain buffer is used to add the same volume of buffer to a vaccine antigen vial for the group receiving antigen alone without the adjuvant.</p> | |
| Purpose of trial | To investigate intra- and inter-subject variability in efficacy of sublingual immunisation with antigen to induce systemic and genital mucosal immunity in healthy female volunteers with or without an adjuvant | |
| Products Administered | <p>Group 1. Sublingual administration on 3 occasions by instillation of drops of antigen mixed with an adjuvant</p> <p>Group 2. Sublingual administration on 3 occasions by instillation of drops of antigen alone, without adjuvant</p> | |
| Key Parameters | <p>1. Anti-antigen IgG and IgA antibody in cervico-vaginal secretions</p> <p>2. Serum anti-antigen IgG and IgA antibody</p> | |
| Product Classification | Sublingual antigen | IMP |
| | Sublingual adjuvant | IMP |
| | Plain buffer solution | IMP |
| Rationale for Clinical Trials Directive status | <p>This study is being conducted by the manufacturer of a licensed vaccine to determine whether sublingual delivery with an adjuvant is better at inducing genital tract immunity than sublingual delivery without adjuvant.</p> <p>As sublingual delivery of the vaccine antigen is being presented as an investigational medicinal product, this is a Phase 1 clinical trial within the scope of the Clinical Trials Directive.</p> | CTIMP |
| Rationale for GMP Directive status | Vaccine antigen for sublingual delivery: as this is a clinical trial it must be made to GMP. | MA(IMP) |
| | Sublingual adjuvant: as this is a clinical trial it must be made to GMP. | MA(IMP) |
| | Buffer solution: as this is a clinical trial it must be made to GMP. | MA(IMP) |
| | As the vaccine components undergo simple mixing of fixed volumes at the bedside, with no more than 2 components being mixed, no manufacture takes place and no IMP(MA) is needed by the clinical site | No MA(IMP) |
| Rationale for required GxP | As this is a clinical trial, full GxP are required SUSAR reporting required | GCP, GPvP, GLP/GCLP |

Table of Abbreviations and definitions

Medicinal Product: substance or combination of substances which either prevent or treat disease in human beings, or are administered to human beings with a view to making a medical diagnosis, or to restore, correct or modify physiological functions in humans.

IMP: Investigational Medicinal Product: defined in Directive 2001/20/EC, Article 2 (d), as “a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form.

NIMP: Non-Investigational Medicinal Product. Medicinal products that do not fall within the definition of investigational medicinal products in Directive 2001/20/EC, such as concomitant or rescue/escape medication for preventive, diagnostic or therapeutic reasons, and/or to ensure that adequate medical care is provided for the subject in a study. They may also be used in accordance with a protocol to induce a physiological response ("challenge agents") where the purpose is to study the physiological response and not the NIMP. The sponsor provides details of NIMPs and their proposed use in the study, and ensures that they are of the necessary quality for human use.

Challenge Agents are defined in Chapter 3 of Volume 10 of the publications “The rules governing medicinal products in the European Union” which contains guidance documents applying to clinical trials [search online for “EUDRALEX Chapter 10”]. The definition places challenge agents in the context of using an IMP, but obviously no IMP need be included: *“challenge agents are usually given to trial subjects to produce a physiological response that is necessary before the pharmacological action of the IMP can be assessed. They may be substances without a Manufacturer's Authorization [i.e. “not licensed”], however some have a long tradition of clinical use”*. The concept that a CA is not an IMP, but used to provoke a physiological response, is established. Eudralex quotes the example of *“open-label sensitivity test of blood pressure response to oral tyramine following treatment with an IMP (new MAO inhibitor) in healthy volunteers.”* The tyramine is a challenge agent, not an IMP. Tyramine given alone, to induce some MAO-related physiological effect, would also be a challenge agent.

Clinical Trial: an investigation in human subjects which is intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of one or more medicinal products, identify any adverse reactions or study the absorption, distribution, metabolism and excretion, with the object of ascertaining the safety and/or efficacy of those products. This includes pharmacokinetic studies. Clinical Trials are regulated by the Clinical Trials Directive 2001/20/EC.

CTIMP: Clinical Trial of an IMP: CTIMPS are regulated by the Clinical Trials Directive 2001/20/EC.

Non-CTIMP: non-clinical trial of an IMP: a study that does not involve investigating the safety and/or efficacy of a medicinal product or substance,

encompassing a variety of research involving NHS and/or non-NHS settings, which may include NHS participants (e.g. patients, patient carers) and/or healthy volunteers. Examples include: questionnaires/interviews; working with human tissue samples and/or data; imaging studies; mechanistic / physiological / experimental medicine studies involving healthy volunteers and/or patients; studies involving the use of CE-marked medical devices or products. Non-CTIMPS are not regulated by the Clinical Trials Directive 2001/20/EC.

Clinical Studies: involving only medical devices, food supplements or other non-medicinal therapies (such as surgical interventions) are not covered by the Clinical Trials Directive 2001/20/EC.

Non-interventional trials: where no additional diagnostic or monitoring procedure is applied, and epidemiological methods are used for the data analysis, are not covered by the Clinical Trials Directive 2001/20/EC.

CTA: Clinical Trial Authorisation: approval by a national regulator to conduct a Clinical Trial.

MA(IMP): IMP Manufacturer's Authorization: permit for the manufacture, assembly, and/or importation of an IMP from third countries, outside the EU.

GxP: umbrella term to include multiple "Good Practices", e.g. GCP, GMP etc

GMP: Good Manufacturing Practice: that part of quality assurance which ensures that medicinal products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorisation (MA) or product specification. GMP is concerned with both production and quality control.

GCP: Good Clinical Practice: a set of internationally recognised ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects. The principles of good clinical practice are outlined in articles 2 to 5 in the EU Directive 2005/28/EC. ICH Topic E 6, the ICH Note for Guidance on Good Clinical Practice is an international standard for GCP. Compliance with this good practice provides assurance that the rights, safety and well-being of trial subjects are protected, and that the results of the clinical trials are credible and accurate.

GPvP: Good Pharmacovigilance Practice: collecting, monitoring, researching, assessing and evaluating information from healthcare providers and patients on the adverse effects of medicines, biological products, herbals and traditional medicines with a view to identifying information about potential new hazards and preventing harm to patients.

GLP: Good Laboratory Practice: a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. GLP helps assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments.

GCLP: Good Clinical Laboratory Practice: analogous to GLP, but specifically a quality system for laboratories which undertake the analysis of samples from clinical trials.

Phase IV Clinical Trials: are carried out after a medicine has been licensed, put on the market and prescribed to patients. Part of the monitoring process, these trials are designed to find out more about the long term harms and benefits of a medicine, and to discover new uses for it. In the UK the requirements for application to conduct a Phase IV trial are considerably less than for earlier phases, consisting of a covering letter, an application form, protocol, and only a "Summary of Product Characteristics (SmPC)" rather than a complete dossier on the IMP. No Investigator Brochure is required and the fee is considerably reduced. However, full GxP are required, SUSARS must be reported, and the trial will be subject to the Clinical Trials Directive 2001/20/EC.

SUSAR: Suspected Unexpected Serious Adverse Reaction

OCP: Oral Contraceptive Pill.

HPV: Human Papilloma Virus

TBE: Tick Borne Encephalitis

MRI: Magnetic Resonance Imaging