# GM Microorganism Risk Assessment

## *Full Assessment, Old Format*

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**Final Classification of Project:** **Class 2**

**Title:** **Role of ubiquitin and ubiquitin-like proteins in transcriptional regulation - replication competent adenovirus work**

**Lab:** M Floor south
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### 1: Brief Description of Project

Ubiquitin and ubiquitin-like proteins are covalently linked to lysine side chains in target proteins and confer altered properties on the modified proteins. Ubiquitin, NEDD8 and SUMO all have important roles in vivo and are required for normal cell growth and division in lower and higher eukaryotes. In lower eukaryotes a single SUMO gene is expressed, whereas in vertebrates three paralogues, designated SUMO-1, SUMO-2 and SUMO-3 are expressed. The conjugated forms of SUMO-2 and SUMO-3, only differ from one another by three N-terminal residues and form a distinct sub-family known as SUMO-2/-3 that are 50% identical in sequence to SUMO-1. Proteomic analysis has indicated that there are a large number of SUMO substrates and demonstrated paralogue specific modification. Many of the SUMO modified proteins identified appeared to be involved in transcriptional regulation, chromatin organisation and RNA metabolism

SUMO is linked to substrate proteins by an enzymatic cascade involving a SUMO activating enzyme (SAE1/SAE2), a SUMO conjugating enzyme (Ubc9) and usually a SUMO protein ligase (E3). Like most other Ubls, SUMO paralogues are synthesised as larger precursors that must be processed to reveal the C-terminal glycine residue that is linked to lysine side chains in target proteins. This processing is carried out by SUMO specific proteases that also remove SUMO from modified substrates and deconjugate polySUMO chains. SUMO modification of transcription factors can lead to transcriptional activation but is more often associated with transcriptional repression. Thus the sites of SUMO modification have been mapped to previously identified repression domains in transcription factors, and mutation of lysine acceptor residues results in increased transcriptional activity. While a considerable body of knowledge has now accumulated on the mechanisms of SUMO modification and deconjugation, there is very little know on the proteins that mediate SUMO dependent responses and there is even less know on how the extent of SUMO modification is regulated. We have determined that SUMO-2 is the paralogue important for transcriptional repression and that stress responses such as hypoxia, heat shock and arsenic treatment induce the conjugation of polymeric chains of SUMO-2 to many substrates. This increase in SUMO-2 modification appears to be a consequence of SUMO protease inhibition. The modified substrates have been identified by proteomics and include many proteins participating in the control of gene expression. Using this information we will identify mediators of SUMO dependent transcriptional repression, determine the mechanism of stress-induced SUMO protease inhibition and establish the structural basis for SUMO protease

specificity.

To achieve these goals, adenoviral based vectors are being used to achieve suitable transfection efficiencies. As some of the genes involved have the potential to regulate the cell cycle, apoptosis and DNA damage responses it is absolutely necessary to minimise the risk to both the environment and those involved in the work.

The major risk to those involved in the project is in the form of aerosol inhalation during manipulation. This is minimised by the use of class 2 microbiological safety cabinets that are annually serviced and KI tested to ensure operator protection. Also, during centrifugation steps executed outwith the cabinet, aerosol containment canisters are employed.

Environmental risk comes from the waste produced by the work. All solid and liquid waste is inactivated by autoclaving on site.

**2: Hazards to Human Health**

A. Identification of potential harmful properties, and consideration of their severity and likelihood of occurrence.

**Adenoviral Vector:**
The adenoviral vector of choice is the replication competent, wild type Ad5 vector. Ad5 is mainly associated with mild respiratory infections, but also has a tropism for ocular and gastrointestinal epithelial cells. Adenoviruses are categorised by the ACDP as Hazard Group 2. Certain adenovirus serotypes have an oncogenic effect in rodents but Ad5 is non-oncogenic in rodents and there is no evidence of adenoviral induced oncogenesis in humans.

**Inserts:**
The constructs to be used in this study, based on the SUMO pathway and associated proteins have not been reported to cause immortalisation or transformation in human cells. The effects of over-expressing some of the various SUMO pathway gene products have not been previously characterised in terms of immortalisation or transformation, however. It is possible, therefore, that changing the expression of genes from this pathway could be harmful. The inhibition of the SUMO pathway could result in activation or inhibition of pathways that regulate the cell cycle and apoptosis, i.e. they could potentially be oncogenic. SUMO regulated transcription factors such as NF-kappaB also regulates inflammation and immune function. It is also possible that expression of these proteins could therefore produce adverse inflammatory effects and immune suppression.

Many transcriptional regulatory proteins have been expressed in adenoviral systems in other laboratories with no known harmful effects on the researchers. In my laboratory we have previously generated an adenovirus expressing a dominant negative form of IkappaB alpha, which strongly inhibits activation of the NF-kappaB pathway (1). Similarly a dominant negative form of the RelA(p65) subunit has also been expressed using adenovirus (2).

**Recombinant Adenoviral Vector:**
The inserts described above do not attenuate the virus in any way, therefore, the resulting recombinant viral vector would be equivalent to the unmodified parent in terms of hazard status, i.e. ACDP Hazard Group 2, thereby warranting Level 2 Containment.

SUMO pathway proteins are not expected to have any effect on the host range or tropism of the virus, since they do not encode proteins that are cell receptors or extracellular matrix proteins, etc.

**Host cells that will be infected with the recombinant adenovirus:**

Well Characterised and Authenticated Tissue Culture cell lines, e.g. HeLa, U2OS, THP-1, MDA-MB-231, MCF7, HT1080 and H1299. These lines are used principally to study the role of the SMUO pathway in cell survival, cell growth and regulation of gene expression. Protein and RNA

extracts will be made from adenovirus infected cells and analysed in various ways in the laboratory. Proliferation and apoptosis assays will also be performed.

Adenovirus is epichromasomal, therefore, there is no integration into the host genome. This removes the potential for activation or inactivation of host genes that could manifest harmful properties not apparent in the unmodified recipient.

B. Assign a provisional Containment Level.

○ Class 1/Level 1 Containment ◉ Class 2/Level 2 Containment ○ Class 3/Level 3 Containment

C. Nature of Work

Brief description of nature of work (include maximum culture volumes)

Standard tissue culture techniques will be applied.
Culture volumes up to 6 litres.

Additional containment measures for work of this nature

**3: Hazards to the Environment**

In the very unlikely event of a release from the Containment Level 2 Facility, the transfected cells would not survive outwith the culture medium ruling out any possibility of survival, establishment or dissemination in the external environment. The adenovirus adenovirus itself is relatively resistant to desiccation and stress, reported as surviving on environmental surfaces for 3-8 weeks. Wild type Ad5 is not known to naturally infect other animals.

**4: If risks identified in Part 3 are not effectively zero specify additional containment measures require to reduce all risks to effectively zero.**

**5: Waste Disposal**

Adenoviruses are sensitive to heat >56°C.
Virkon is effective at 1% for 10 min (efficacy data available on manufacturer's web site) and will be used for surface disinfection.

Liquid waste is collected in a sealable, robust, autoclavable container, autoclaved then disposed of to drains. If using a microbiological safety cabinet store the container within the cabinet during use and seal before removal. Aspirator set-ups are not used.

Solid waste (including plastic pipettes and agar plates) is collected in a lined, biohazard labelled, autoclavable bin, autoclaved then disposed of as normal refuse.

Large glass pipettes are not used.

Sharps waste is collected in an autoclavable sharp-safe container, autoclaved then disposed of as clinical waste. If using a microbiological safety cabinet store the container within the cabinet during use and engage temporary closure before removal. The use of sharps must be avoided unless essential.

**6: Validation and Monitoring**

<u>Autoclave Testing and Maintenance</u>
During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced every 6 months by a reputable service provider and calibrated annually to ensure the validation criteria are met. During normal, daily operation indicator tape and, in the case of liquid waste, a

temperature probe placed at the centre of the load, are used to ensure the required conditions are achieved. Servicing and testing is arranged and test reports are kept by the CLS Health & Safety Coordinator.

Maintaining PPE

Users are required to routinely check their PPE (e.g. lab coat, safety glasses) and keep it in good order. Defective PPE must be repaired or replaced immediately. Laboratory Managers are required to ensure the appropriate PPE is readily available and keep an inspection record for non-standard PPE, e.g. that used in Liquid Nitrogen facilities.

Inspections, Audits and Continual Monitoring

Safety Inspections are carried out regularly to ensure health & safety policy & procedures are being followed and that the required risk assessments and training records are complete and up to date. Inspections are timetabled and inspection teams selected by the CLS Health & Safety Working Group. Inspection team members are selected from CLS Health & Safety personnel and senior management. Inspection reports are submitted to the CLS Health and Safety Management Committee for review. Audits performed by an external, independent body are arranged by the CLS Health & Safety Working Group when deemed necessary by the CLS Health & Safety Management Committee. Lab Managers and Biological Safety Advisers are required to continually monitor safety standards and compliance with Health & Safety Policy & Procedures, within their designated area, and report problems and non-compliance to the CLS Health and Safety Working Group.

MSC Testing and Maintenance (TC suites only)

MSCs are serviced and operator protection (KI) tested on an annual basis by a reputable service provider. A certificate of conformity to the required standard is displayed on each cabinet. The Health and Safety Information Officer is responsible for arranging the servicing schedule, ensuring fumigation/decontamination is carried out prior to testing and issuing and keeping a copy of the certificates of conformity. Users are required to perform a visual check on all alarms and indicators before each use and report any defects immediately to their Lab Manager. Note: If an MSC is moved to a new location, or equipment in a room containing a cabinet is significantly re-arranged, to the extent where it may affect the airflows within the room, the cabinet must be KI tested before use to ensure operator protection has not been compromised.

Negative Pressure Testing (TC suites only)

Pressure differentials in TC suites are checked regularly to ensure the suite is at an air pressure negative to the immediate surroundings. Checks are arranged by the CLS Health & Safety Information Officer.

**7: Storage and Transport**

Samples must be doubly contained during transport within/between buildings & clearly labelled with a contact name & number, the nature of the sample & the biohazard symbol. Inner container/tube must be robust & leak-proof. Outer container must be robust, leak-proof & contain enough absorbent material to absorb the total volume of sample should the inner container leak.

Samples in liquid nitrogen cryo-stores must be contained in proper cryo-tubes & stored in the liquid nitrogen vapour phase to eliminate risk of tube explosion upon initial warming. Samples stored in fridges/freezers must be doubly contained. Fridges, freezers & cryogenic storage vessels must be secure, biohazard labelled & subject to a well maintained inventory system.

**8: Emergency Plan**

Required? ○ Yes ◉ No

If not required for Class 2 or 3 activities give reason:

Activity is small scale and risk is low.

**9: Health Surveillance**

Required? ○ Yes ◉ No

If yes, give details:

**10: License required? (animal, fish, bee and plant pathogens only)**

Required? ○ Yes ◉ No

**11: Final Classification of project**

○ Class 1 ◉ Class 2 ○ Class 3

**12: Additional Information and Comments**

**13: References**

1. Clarkson RW, Heeley JL, Chapman R, Aillet F, Hay RT, Wyllie A, Watson CJ. NF-kappaB inhibits apoptosis in murine mammary epithelia. J Biol Chem. 2000 Apr 28;275(17):12737-42

2 Soares (1998). “Adenovirus-mediated expression of a dominant negative mutant of p65/RelA inhibits proinflammatory gene expression in endothelial cells without sensitizing to apoptosis”. J. Immunol.*161*, 4572-4582