Loughborough University **Biological Risk Assessment**	Safety Department use only		Material(s) Classification	
	Reference Number:		Hazard Group 1	☑
			Hazard Group 2	☐
	CBE Use only		GMO	☐
	Reference Number:	CBE BRA 198	HTA Licensable	☑

FORM CBE-RA-Form/002 Version 1.0

RISK ASSESSMENT AND PROJECT REGISTRATION FOR WORK INVOLVING BIOLOGICAL MATERIAL

PLEASE READ CAREFULLY

This form acts to register projects involving the use of Biological Agents and / or Genetically Modified Micro-Organisms, or of materials that may be contaminated with these agents. It assesses the hazards and risks associated with the project as well as identifying those at risk and the measures necessary for preventing, or controlling these risks. Please ensure that sufficient detail is provided when completing this form and that the relevant written SOPs are referenced where required. Once completed and approved, all risk assessments must be supplied to all those working within this project. The work described within this form must not commence until this risk assessment has been completed and approved and that all necessary control measures are in place.

Any changes to the work, or the persons involved, must be notified to the authorised person. All changes requested must be recorded within the risk assessment change control form and may also need to be incorporated within an amended version of this form.

A separate risk assessment will be required for assessing risks associated with GMO activities.

The following declaration must be completed and undersigned by the Principal Investigator or Person Responsible for the project

- All information contained in this form is accurate and comprehensive.
- All workers involved will be instructed that their work must remain within the boundaries of this project registration & assessment.
- All workers have been given, or will be given before they become involved, adequate training and where necessary their competency assessed.
- All workers have, or will be before their involvement begins, enrolled with Occupational Health for health clearance where necessary.
- It is understood that this risk assessment shall not be transferred to a third party without the PI/Supervisor/Line Manager named in this form either taking responsibility for the new activities, or ensuring that a new proposal is submitted.
- All changes to the work covered by this form will be reassessed & the changes submitted to the authorised person before those changes are made to the work.

Principal Investigator		Person conducting this risk assessment	
Name	Dr Karen Coopman	Name	Nishant Joglekar
Position	Reader	Position	PhD student
Department	Chemical Engineering	Department	Centre of Biological Engineering
School	AACME	School	AACME

The Project Activity			
Title	Ordering primary CD4+ T cells and co-culturing with MSCs or culturing T cells in MSC conditioned medium (CM-MSC)		
Reference Number			
Start Date	3 Mar 2021	End Date	Open ended

Others involved in the work	
Names	Jen Bowdrey
	Technician
	Centre of Biological Engineering
	AACME

Name	Nishant Joglekar	Signature	Nishant Joglekar Digitally signed by Nishant Joglekar Date: 2021.03.03 11:32:36 Z	Date	3 Mar 2021

1. INTRODUCTION

1.1 Background & aim of project	The aim of this work to investigate the ability of MSCs post-thaw, and in culture, to be able to differentiate CD4+ T cells into T regulatory cells (Tregs) following co-culturing the T cells with MSCs or culturing T cells in MSC conditioned medium (MSC-CM)
1.2 Description of experimental procedures	Thawing cells - refer to CBE BRA 183 Freezing cells - refer to CBE BRA 183 Culturing MSCs - MSCs will be cultured at a density of 5000cells/cm^2 in DMEM medium containing 10% FBS and 2uM ultraglutamine. Media will be changed every three days. Culturing T cells - T cells will initially be thawed and cultured in flasks/well plates in order to make a working cell bank (WCB). The cells from the WCB will then be used for further experiments with MSC-CM, or for co-culture with MSCs. T cell culture will involve an RPMI based medium containing 10% FBS, or ImmunoCult™-XF T Cell Expansion Medium with an appropriate amount of ImmunoCult™ Human CD3/CD28 T Cell Activator and human rIL-2 to activate the cells before transferring the flasks/plates to a 5% CO2 incubator at 37C. Cells will be seeded at 1x10^6cells/ml post-thaw, and samples will then be examined daily with cell counts taken every 24hrs. Cell suspension will be mixed with a pipette every 3 days to remove clumps. If cells reach a concentration of 2.5x10^6cells/ml, or the media turns yellow, fresh media will be added to adjust the concentration back to 1x10^6cells/ml. If longer term cell expansion is required (>12days), fresh activator and IL-2 will be added for restimulation. MSC and T cell co-culture - MSCs will initially be cultured in 24 well plates in DMEM medium. At 80-90% confluency, the media will be aspirated and T cells cultured for 24hrs with RPMI based medium containing 10% FBS, or ImmunoCult™-XF T Cell Expansion Medium with an appropriate amount of ImmunoCult™ Human CD3/CD28 T Cell Activator and human rIL-2 . MSCs used will either be post-thaw or post-passage. The T cells will then be inspected each day and samples taken to determine cell number and viability, along with the number of Tregs present using the Treg detection kit. Preparation of MSC conditioned media (MSC-CM) - MSCs will initially be cultured in DMEM containing 10% FBS till 80-90% confluent. DMEM media will then be aspirated and appropriate serum free media (SFM) will be added and the cells cultured in SFM for 72hrs. Following the 72hr incubation period, media will be harvested, filtered to remove any debris, and will be concentrated using a centrifugal filter device. The prepared MSC-CM will then be stored at -80C until further use. MSCs used to prepare MSC-CM will either be post-thaw or post-passage. Culturing T cells in MSC-CM - T cells will initially be cultured in RPMI based medium containing 10% FBS, or ImmunoCult™-XF T Cell Expansion Medium with an appropriate amount of ImmunoCult™ Human CD3/CD28 T Cell Activator and human rIL-2 in 24 well plates for 24hrs. T cells will then be harvested using centrifugation, and re-dispersed in the previously prepared MSC-CM and fresh ImmunoCult™ Human CD3/CD28 T Cell Activator and IL-2 will be added. The T cells will then be inspected each day and samples taken to determine cell number and viability, along with the number of Tregs present using the Treg detection kit. Treg identification - Following MSC co-culturing/incubation in MSC-CM, it is likely that the CD4+ T cells will differentiate into T regulatory cells (Tregs). At various timepoints, T cells will be harvested, and the number of Tregs identified using flow cytometry using a Treg detection kit - involves staining for CD45+/CD4+/CD25+/CD127dim/neg/FoxP3+ cells.
1.3 Where will this work be carried out?	Rooms/areas: H23, H30, H34 - Centre for Biological Engineering (CBE)
	Building(s): CBE

☑ **2.1 Human or animal tissues, cells, body fluids or excreta will be used in this project**

2. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

2.2 List all cells, tissues, body fluids and excreta to be used. For cells, indicate primary, continuous or finite.

Material type	Organ source	Species	Where it will be obtained from (Include country of origin)	+
Primary cells (CD4+ T cells)	Peripheral blood	Human	Stemcell Technologies, UK	x

☑ **2.3 Material(s) listed in section 2.2 above are considered to be 'relevant material' under the Human Tissue Act 2004.**

Government Human Tissue Authority - Web Page

2.3.1 Relevant material type	**Source / Provider** *A = Commercial provider* *B= HTA licensed Biobank with REC approval for genetic research use* *C = Other* *D = Organisation with REC approval for research use* *E = Imported*	+

Commercial provider	☑ A	☐ B	☐ C	☐ D	☐ E	Stemcell Technologies	x
2.3.1.1 Has a Material Transfer Agreement (MTA) been fully approved?	○ Yes ◉ No						
2.3.2 Have you verified that the consent has taken place for use of tissue in this study?	◉ Yes ○ No	Give details:	Company has generalised consent in place				
2.3.3 Are you aware of the Ethics expiry date?	◉ Yes ○ No	Expiry Date:	Provided by company				
2.3.3.1 Please detail the sample disposal action plan.	Disposal plan at CBE includes signing a disposal form if still HTA relevant material after initial passaging, and taking item off ProCuro. In the case of disposing HTA-relevant cells, an 'Authorisation to Dispose of HTA Licensable Material form' must be completed and submitted to the PI (or Person Responsible) and dPD for approval in advance of the proposed date for disposal - refer to CBE/HTA-PR-SOP007 for further details.						

☐ 2.11 Biological agents will be used in this project

3. CLASSIFICATION OF HAZARD GROUP

3.1. Are you confident that any non-GM organism, tissue, cell, body fluid, excreta or any component thereof covered by this assessment cannot potentially pose a threat to humans or cause human diseases?	◉ Yes - Classify as HG1
3.1.1. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause human disease and potentially be a hazard to humans but is unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available?	○ Yes - Classify as HG2
3.1.2. Can any non-GM organism, tissue, cell, body fluid, excreta or any component thereof cause severe human disease and potentially be a serious hazard to humans and that may spread to the community, where effective prophylaxis or treatment may or may not be available?	○ Yes
3.2. Do any of the materials contain pathogens or toxins covered by the Anti-Terrorism Crime and Security Act?	○ Yes **ATCSA Schedule 5**
ASSIGNMENT OF CONTAINMENT LEVEL	HG1

4. TISSUES, CELLS, BODY FLUIDS OR EXCRETA

4.2. Will any culturing of the material described in section 2 take place? *If Yes, describe which cell(s) will be cultured and under what conditions.*	◉ Yes ○ No	T cells will be cultured in 24 well plates/T flasks in an incubator at 37C
4.3. Could HIV permissive cells be present*? *If Yes, describe the cells and for how long these cultures will be allowed to grow.* *If unsure seek advice. Refer to CBE Code of Practice for details on additional precautions.*	○ Yes ◉ No	
4.4. What is the maximum volume of culture grown?	Per Vessel	6,000,000
	Number of vessels	10
4.5. Will the tissues, cells, body fluids or excreta be manipulated in any way that could result in the concentration of adventitious biological agent present? *If Yes, explain.*	○ Yes ◉ No	
4.6. Will any of the tissues, cells or fluids be donated by you or your colleagues working in or with access to the labs?	○ Yes ◉ No	

5. RISKS AND CONTROL MEASURES

Risk		How will this be controlled?	Reference to SOP's / Other documentation

Risk		How will this be controlled?	Reference to SOP's / Other documentation
5.1. Might infectious droplets, aerosols or splashes be created, either deliberately or by accident?	☑ Yes ☐ No	Cell culture will be carried out in ClassII BSCs using aseptic technique. If spills occur, the spill procedure as outlined in the SOP will be followed. PPE will be worn at all times while working in the labs. Possible small spillages of ICF and bleach used during flow cytometry will be cleaned as per the procedure outlined in the risk assessment for the 'Use and maintenance of Guava easyCyte 8HT benchtop flow cytometer'	SOP037 SOP038 CBE 186 SAF MEME 6698
5.2. Will this material be transported within the laboratory e.g. between BSC & incubator?	☑ Yes ☐ No	24 well plates/T flasks will be transported between the BSC and incubator using due care and diligence. This will include making sure that lids are properly closed to prevent spillages and also infection of cells. Making sure that there are no trip hazards present in the lab before work begins.	
5.3. Will this material (including waste) be transported locally between sites on campus but outside the laboratory?	☐ Yes ☑ No		
5.4. Will material(s) listed in section 2.2 or section 2.3 be shipped to organisations elsewhere in the UK or abroad?	☐ Yes ☑ No		
WHO guidance for transport of infectious substances website			
5.5. Will this material be received from organisations elsewhere in the UK or abroad?	☑ Yes ☐ No	Cells will be supplied from a commercial supplier within the UK.	
5.6. Will this material be stored?	☑ Yes ☐ No	Cells will be stored in the vapour phase of liquid Nitrogen. Correct PPE will be worn when transferring cells to and from liquid nitrogen as per the SOPs. When in culture the cells will be stored in incubators at 37C and 5% CO2.	SOP031 SOP013
5.7. Will infectious material be centrifuged?	☑ Yes ☐ No	Both MSCs and T cells will be centrifuged at various points in the culture process.	SOP047
5.8. Are biological samples to be cultured in an incubator?	☑ Yes ☐ No	Cells will be cultured in an incubator under standard conditions. Temperature and CO2 levels will be regularly checked and any irregularities will be reported to the lab manager. Incubators used as per the SOP114. If spills occur they will be cleaned up with 70% IMS, or if large as per the SOP038	SOP114 SOP038
5.9. Are sharps to be used at any stage during this activity?	☑ Yes ☐ No	Pipette tips maybe used duiring cell culture. These will be placed in the yellow autoclable sharps containers for safe disposal. Sharps containers will not be filled more than three quarters full. If a sharp injury occurs, the wound will be washed immediately and the lab manager, first aider and safety officer are informed. A near miss/accident form also needs to be completed.	SOP003
5.10. Are animals to be used in this project?	☐ Yes ☑ No		
5.11. Will a fermenter / bioreactor be used to culture a biological agent or material?	☐ Yes ☑ No		
5.12. Is there any stage within the experimental procedures when an infectious material is inactivated (other than for disposal)?	☐ Yes ☑ No		
5.13 Are any of the following to be used in conjunction with the project?	☐ Carcinogens or Mutagens		
	☐ Toxins		
You must complete a cryogen risk assessment before work begins and add the reference here.	☑ Liquid Nitrogen	Used for storage of cells.	SOP013
	☐ Ionising radiation		

Risk		How will this be controlled?	Reference to SOP's / Other documentation
You must complete a lone working risk assessment before work begins and add the reference here.	☑ Lone working	Media changes/passages might be performed on the weekend.	SAF/MM/6576
5.14. Are there any conditions associated with the hazards described in section 5.13 that require additional control measures?	○ Yes ☑ No		

6. PPE AND HYGENE

Control Measure	Details		Reference to SOPs / other documentation
6.1 When will gloves be worn?	At all times while in the lab		SOP037
6.2 What type and where will they be stored?	Nitrile	In Lab and in Changing Area	SOP037
6.3 When will laboratory coats be worn and what type are these?	At all times in the lab	White Howie	SOP037
6.4 Where will lab coats be stored and what are the arrangements for cleaning or disposal?	Stored in first change	Lab coats are cleaned once a month, or if they come into co	SOP037
6.5 Provide details of any other types of PPE to be used?	Shoe covers are to be worn at all times over closed toe shoes. Safety glasses to be worn when appropriate. Heavy duty gloves will be worn during handling the cryobanks.		SOP037
6.6 Describe the lab hygiene facilities available and where they are located	Sinks and eye wash stations	In change areas and labs	SOP037
6.7 Where are the first aid boxes and emergency spill kits located?	First aid kit - Office and First c	Spill kits- In first changes, and under hand sinks	

7. WASTE

7.1 How will waste be treated prior to disposal

(Note that all differently treated wastes must be included e.g. if some liquid is autoclaved, but others not, then describe both)	Treatment prior to disposal	Is the treatment validated?	Reference to SOPs / other documentation
☑ Liquid waste	Liquid waste can be autoclaved or treated with Virkon for 24 hours then discarded down the drain with copious amounts of water	☑ Yes ○ No	SOP003 SOP025
☑ Solid waste	Solid waste contaminated with biological agents which does not contain chemicals can be autoclaved on cycle 4, and discarded via the orange waste stream. If solid waste contains and disinfectant or chemicals, this must be disposed of via the yellow waste stream.	☑ Yes ○ No	SOP003 SOP025
☐ Other (Specify)			
7.2 Is any waste being autoclaved?		☑ Yes ○ No	SOP003 SOP025
All cycles have been validated for the actual load types used? *(If Yes, documentary evidence of the validation must be available)*		☑ Yes ○ No	SOP054
The successful completion of every load is checked prior to disposal?		☑ Yes ○ No	SOP054

7.3 How will liquid waste be disposed of?

☑ To drain?	Autoclaved or virkon treated liquid waste can be poured do	☑ Yes ○ No	SOP003 SOP025

7. WASTE

☐ As solid waste?			
☐ Other (Specify)			

7.4 How will solid waste be disposed of?

Categorisation	Waste stream colour code	Disposal method (Edit as required)
☑ Sharps	**Orange**	Yellow/Orange lidded sharps bin > autoclave sterilisation if known or potentially infected > clinical waste disposal (incineration)
☐ Sharps contaminated with cytotoxic or cytostatic material		
☐ Human body parts, organs, including blood bags and blood preserves and excreta that have been pretreated before leaving the site		
☐ Animal body carcasses or recognisable parts that have been pretreated before leaving the site		
☐ Potentially or known infected lab wastes contaminated or potentially contaminated with cytotoxic or cytostatic material that have **NOT** been pretreated before leaving the site		
☑ Potentially or known infected lab wastes that have **NOT** been pretreated before leaving the site	**Yellow**	Yellow clinical waste bags > clinical waste disposal (incineration)
☑ Infected or potentially infected lab wastes that **HAVE** been pretreated before leaving site	**Orange**	Disinfection or sterilisation in the lab site > orange clinical waste bags > clinical waste disposal (incineration)
For HTA: Please specify how you will ensure segregation of tissue from the deceased from other clinical waste.	The procuro system will be used to keep track of where HTA relevant material is at each stage of the process and when disposing, the HTA relevant material will be collected in separate containers/bags marked for disposal of HTA Licensable Material to ensure that the material is kept separate from non-human and other laboratory clinical waste. Details of disposing HTA relevant material have been provided in CBE/HTA-PR-SOP007.	

8. MAINTENANCE

8.1 Are preventative maintenance and monitoring regimes in place for the following laboratory equipment?

	Inspection / Servicing Frequency	Cleaning / Disinfection Frequency	Monitoring / Alarms Frequency	Reference to SOPs
☑ Centrifuges	User inspection before each use. Weekly checks. Serviced after 100-150 hours use.	Weekly	With each use	SOP047
☑ BSCs	Weekly	Before and after use, also a weekly clean	Before each use. Record values on BSC daily use sheet.	SOP004
☐ Fume Hoods				
☑ Autoclaves	Inspected before each use	Weekly	Before each use check Autoclave in safe working condition. Check cycle has worked after each run. Check display for error messages.	SOP025
☑ Incubators	Weekly	Fornightly/monthly/ when required	With each use	SOP114

8. MAINTENANCE				
☑ Liquid N_2 Stores	Biweekly	Biweekly	Biweekly	SOP013
Failure contingency plan	In the event of failure or malfunction, the dRP with the custodians of the stored material shall transfer material to an available space within another cryostore. Transfer shall be recorded on the Procuro database. In the event of unplanned long term facility/utility failure the dRP shall seek permission with the relevant authority to transfer the cryostore to another facility to allow continued maintenance. Transfer shall be recorded on the Procuro database. In the event of alarm/probe failure, the dRP shall ensure that the alarm/probe is repaired.			
☑ Freezers	Biannually	Biannually	Biannually	SOP016
Failure contingency plan	For -80C freezer: In the event of failure or malfunction, the dRP with the custodians of the stored material shall transfer material to an available space within another CBE -80C freezer or to an available space within a cryostore. Transfer shall be recorded on the Procuro database. In the event of a short-term power outage, all freezers in the CBE will be affected. To minimise the effect stored material, the freezer should not be opened during the power outage as well as an hour after power returns to allow the freezer to stabilise. The temperature log should be reviewed to assess the impact on the freezer contents. In the event of a planned long term power outage, the dRP shall ensure that there is alternative source of power (generator) hired in for the duration of the power outage. In the event of unplanned long term power outage the dRP shall seek permission with the relevant authority to transfer material to the SSEHS facility. Transfer shall be recorded on the Procuro database In the event of alarm failure, the dRP ensures that the alarm is repaired and batteries replaced, if applicable. The dRP checks the probes and alarm system regularly and ensures maintenance. For -20C freezer: In the event of failure or malfunction, the dRP with the custodians of the stored material shall transfer material to an available space within another CBE -20C freezer or to an available space within the -80C freezer. Transfer shall be recorded on the Procuro database. In the event of a short-term power outage, all freezers in the CBE will be affected. To minimise the effect on stored material, the freezer should not be opened during the power outage and an hour after power returns to allow the freezer to stabilise. The temperature log shall be reviewed to assess the impact on the freezer contents. In the event of a planned long term power outage, the dRP ensures that there is alternative source of power (generator) hired in for the duration of the power outage. In the event of unplanned long term power outage the dRP shall seek permission with the relevant authority to transfer material to the SSEHS facility. Transfer shall be recorded on the Procuro database. In the event of alarm failure, the RP shall ensure that the remote alarm is repaired and probes replaced.			
☑ Fridges	Biannually	Biannually	Biannually	SOP016

Failure contingency plan	In the event of failure or malfunction, the dRP with the custodians of the stored material shall transfer material to available space within another CBE 2-8C fridge. Transfer shall be recorded on the Procuro database. In the event of a short-term power outage, all fridges in the CBE/T208b will be affected. To minimise the effect on stored material, the fridge should not be opened during the power outage and an hour after power returns to allow the fridge to stabilise. The temperature log shall be reviewed to assess the impact on the freezer contents. In the event of a planned long term power outage, the dRP ensures that there is alternative source of power (generator) hired in for the duration of the power outage. In the event of unplanned long term power outage the dRP shall seek permission with the relevant authority to transfer material to the SSEHS facility. (T208b will use CBE as back up in first instance but use SSEHS if this is not viable). Transfer shall be recorded on the Procuro database. In the event of alarm failure, the RP shall ensure that the remote alarm is repaired and probes replaced.
☐ Others	

9. TRAINING

9.1. Have all project research workers undertaken safety training for working with hazardous or potentially hazardous biological materials and agents at CL2?

Name of researcher	Had Training	Date training completed (or will be completed)	If no, state why	+
Nishant Joglekar	◉ Yes ○ No	15 Oct 2019		x

☑ 9.2. This work involves HTA 'Relevant Material', confirm that all project research workers have undertaken HTA training

		Date training completed (or will be completed)				
Name of researcher	Had Training	Induction	On-line	In-house	If No, state why	+
Nishant Joglekar	☑ Yes ○ No	9 Mar 2021	3 Mar 2021	22 Mar 2021		x
Jen Bowdrey	☑ Yes ○ No	24 Jan 2017	15 Oct 2020	23 Oct 2018		x

10. EMERGENCY PROCEDURES

10.1 Are procedures in place for dealing with spillage of infectious or potentially infectious material

Equipment	Reference to SOPs
☑ Within the BSC	SOP038
☑ Within the centrifuge	SOP038
☑ Within the laboratory, but outside any primary control measures (e.g. BSC)	SOP038
☐ Outside the laboratory	

Are procedures in place for the security of these HTA Relevant samples?

☑ Loss or theft of samples (including whilst in transit)	

10. EMERGENCY PROCEDURES

☑ Loss of traceability of samples	
☑ Incorrect disposal of samples	

10.2 Describe the procedures in place for an accidental exposure +

Immediate action	Skin exposure-flush with running water and wash with soap. Eyes-flush with eyewash for 15 minutes Sharps injury-encourage bleeding and seek medical attention.	Ref to SOP's	SOP038	x
When and whom to report the incident	Contact first aider and report to lab manager and DSO. Complete the	Ref to SOPs	SOP038	

11. ACCESS

		Explanation	References
11. Is/are the lab(s) adequately separated from other areas (e.g. offices)?	◉ Yes ○ No		
11.2. Is/are the lab(s) or other work areas shared with other users not involved in the project?	◉ Yes ○ No	Work areas will be shared with users working on other projects. Other lab users will be informed of the type of work being carried out and alerted to any potential hazards. Work will be carried out in BSCs and any work areas cleaned before and after use.	SOP004
11.3. Describe the measures in place to ensure that hazardous biological agents or **HTA relevant** material is secure	◉ Yes ○ No	Labs can only be accessed and HTA material can only be handled by other authorised users who have undergone safety training.	

12. OCCUPATIONAL

12.1. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunisation. Have all workers involved in this project been immunized?	◉ Yes ○ No
12.2. Is health surveillance required?	○ Yes ◉ No

13. NOTIFICATIONS

☑ 13.1. Are any of the cells, tissues or fluids covered by the Human Tissue Act (HTA) under the University HTA Licence?	Primary CD4+ T cells
☐ 13.2. Are any of the cells, tissues or fluids obtained from a HTA licensed biobank with REC approval for generic research use?	
☐ 13.3. Does this work have ethical approval from a recognised NHS Research Ethics Committee?	
☐ 13.4. Does any of the work require approval from the University Ethical Committee?	
☐ 13.5. Do any of the materials require approval for use from the UK Stem Cell Bank Steering Committee (MRC)?	
☐ 13.6. Do any of the materials or biological agents listed require any other licenses?	

14. APPROVALS

14. APPROVALS	
Authorised Person	Karen Coopman Digitally signed by Karen Coopman Date: 2021.03.23 11:43:18 Z
Departmental Biological Safety Advisor	Carolyn Kavanagh Digitally signed by Carolyn Kavanagh Date: 2021.03.23 13:44:49 Z