BSc Degree Examinations 2019-0

Department:
BIOLOGY

Title of Exam:
Cancer cell and molecular biology

Time Allowed:
24 Hours

Time Recommended:
2 hours 30 minutes

Word limit: Please answer the questions within the line limit stated. Content beyond the line limit will not be marked.

Allocation of Marks:
Total marks available for this paper: 100
The marks available for each question are indicated on the paper.

Instructions for Candidates:
Answer all questions within the line limits stated on the examination paper.
Please use Arial font, size 11 or larger.
Content beyond the line limit will not be marked.
Do not adjust the margin width.
A note on Academic Integrity

We are treating this online examination as a time-limited open assessment, and you are therefore permitted to refer to written and online materials to aid you in your answers.

However, you must ensure that the work you submit is entirely your own, and for the whole time the assessment is live you must not:

- communicate with departmental staff on the topic of the assessment
- communicate with other students on the topic of this assessment.
- seek assistance with the assignment from the academic and/or disability support services, such as the Writing and Language Skills Centre, Maths Skills Centre and/or Disability Services. (The only exception to this will be for those students who have been recommended an exam support worker in a Student Support Plan. If this applies to you, you are advised to contact Disability Services as soon as possible to discuss the necessary arrangements.)
- seek advice or contribution from any third party, including proofreaders, friends, or family members.

We expect, and trust, that all our students will seek to maintain the integrity of the assessment, and of their award, through ensuring that these instructions are strictly followed. Failure to adhere to these requirements will be considered a breach of the Academic Misconduct regulations, where the offences of plagiarism, breach/cheating, collusion and commissioning are relevant - see AM.1.2.1” (Note this supersedes section 7.3 of the Guide to Assessment).
Question 1

Tumour suppressor p53 protects against cancer in multiple ways.

a) Which two hallmarks of cancer are most commonly acquired following loss of wild-type p53 function?  
   (2 lines; 2 marks)

The western blot below compares expression of p53, p21 and actin in five isogenic cell lines with two wild-type p53 alleles (p53+/+), one wild-type allele (p53+/-), no p53 genes (p53-/-), or an R248Q missense mutant p53 allele in the presence (R248Q+/+) or absence (R248Q/-) of a wild-type allele. The cells were treated with (+) or without (-) daunorubicin, as indicated.

b) Explain how these data demonstrate a dominant-negative effect of the R248Q mutant.  
   (4 lines; 2 marks).

c) A screen for dominant-negative effects of p53 mutants used a GFP reporter expressed from the p21 promoter. If an effect was observed, how would you test whether a mutant was acting directly or indirectly?  
   (3 lines; 2 marks).

d) Why do p53 missense mutants commonly accumulate to higher levels than wild-type p53?  
   (4 lines; 2 marks)
e) How does R248Q mutant p53 increase MLL1 expression in breast cancer cells?  

(5 lines; 2 marks)

f) Why does 5-azacytidine increase recruitment of MLL1 to promoters?  

(3 lines; 2 marks)

**Question 2**

Prostate cancer cells were treated with DMSO vehicle (lanes 1 & 2) or 5 μM palbociclib (lane 3). Cell lysates were then immunoprecipitated with control antibody (lane 1) or antibody that recognizes RB (lanes 2 & 3). Below are shown western blots probed with antibodies that recognize p65, E2F1, ERK2, total RB (all phosphorylated forms) or RB phosphorylated on S795, as indicated. The top three panels show blots of immunoprecipitated proteins (IP) as indicated. The other panels show blots of proteins in the input lysates.

![Western Blot Image]

a) What conclusions can be drawn from these data?  

(4 lines; 3 marks)
b) How is palbociclib beneficial to patients with breast cancer? (3 lines; 2 marks)

c) In what way might palbociclib be detrimental to patients? (4 lines; 2 marks)

d) Although p65 was found to co-immunoprecipitate with RB from cancer cells, RB failed to bind to p65 when both were expressed in bacteria. Suggest two likely explanations for this discrepancy. (5 lines; 4 marks)

e) How would tumour suppressor activity be impacted by deletion of the entire INK4a gene? (8 lines; 4 marks)

Question 3

a) Why does sequencing of cancer genomes underestimate the frequency of MYC induction in cancers? (12 lines; 5 marks)
b) Briefly summarise the mode of action of Omomyc. (7 lines; 3 marks)

c) Below are shown western blots for BRD4, MYC and tubulin in xenograft tumour samples from nine mice treated with vehicle or nine mice treated with the PROTAC ARV-771, as indicated. Explain how ARV-771 produces the effects shown below. (10 lines; 4 marks)

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>ARV-771</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRD4</td>
<td></td>
</tr>
<tr>
<td>MYC</td>
<td></td>
</tr>
<tr>
<td>Tubulin</td>
<td></td>
</tr>
</tbody>
</table>

d) What is the most likely explanation for the low BRD4 in lane 3? (2 lines; 1 mark)

e) What are the advantages of PROTACs relative to conventional small molecule drugs? (5 lines; 2 marks)
Question 4

Ovarian cancer cells were transfected with a vector encoding HA-tagged EZH2 wild-type (WT, lanes 1 and 2) or with threonine 311 substituted to alanine (T311A, lanes 3 and 4). The cells were then treated with vehicle (lanes 1 and 3) or with A769662, an agonist for AMP-activated kinase (lanes 2 and 4). Below are shown western blots (IB) for H3K27me3, total histone H3 and EZH2, as indicated.

![Western blot image]

a) What conclusions are suggested by these data? (2 lines; 4 marks)

b) Describe two caveats to these conclusions. (3 lines; 4 marks)

c) Predict the effect of A769662 on transcription of the INK4A gene, a target of EZH2 and justify your prediction. (4 lines; 2 marks)

d) What is the evidence that elevated EZH2 activity can be oncogenic? (6 lines; 4 marks)
Question 5

The diagram below shows the results of an in vitro invasion assay using metastatic lung cancer cells. The effects of the following compounds were tested: a voltage-gated sodium channel inhibitor (TTX), a MEK inhibitor (U0126) and a PI3K inhibitor (wortmannin) and compared to untreated control cells (CTL). Asterisks indicate statistical significance between treatments (and control if no brackets shown). n.s. indicates difference is not significant.

![Diagram showing relative invasion results for different treatments.](image-url)

a) Interpret these data. (8 lines; 4 marks)

b) What additional in vitro experiments could you do to strengthen these findings? (10 lines; 4 marks)

c) Describe an experiment you could perform to test the effect of voltage-gated sodium channels on lung cancer metastasis. (12 lines; 5 marks)
Question 6

The diagram below shows the survival of immunocompetent mice bearing syngeneic orthotopic lung tumours. The mice were treated with/without a selective cyclin-dependent kinase (CDK7) inhibitor (YKL-5-124) or anti-PD-1 antibody, alone or in combination for up to 90 days. Asterisks indicate statistical significance of differences between treatments.

a) What conclusions can be drawn from these data?  
(7 lines; 3 marks)

b) CDK7 inhibition potentiates immune cell infiltration. Suggest a plausible mechanism to explain how CDK7 might regulate anti-tumour immunity. 
(7 lines; 3 marks)
c) Describe an experiment to test your proposed mechanism.  
(12 lines; 4 marks)

d) What additional experimental evidence might be useful before testing this combination in the clinic?  
(7 lines; 3 marks)

Question 7

a) Hormonal therapies are used to treat breast and prostate cancers. Explain the principle of this approach.  
(8 lines; 3 marks)
b) Briefly describe the steps by which prostate cancers acquire resistance to hormonal therapy through lineage plasticity.  

(7 lines; 3 marks)

c) The graph below shows tumour growth in enzalutamide-treated mice after xenograft of prostate cancer cells expressing non-targeting shRNA (shNT), or shRNAs targeting p53 and RB (shTP53/RB1) with or without shRNA targeting SOX2 (shSOX2). Interpret these data.  

(3 lines; 2 marks)

![Graph showing tumour growth in enzalutamide-treated mice](image)

(d) Researchers introduced into breast cancer cells a DOX-inducible shRNA that depletes NF1 mRNA, as shown by RT-PCR (inset below). The graph below shows tumour growth in mice with xenografts of these cells in the presence or absence of DOX and tamoxifen (+ Tam) or vehicle (+ Veh). Interpret these data.  

(6 lines; 3 marks)

![Graph showing tumour growth in breast cancer cells](image)
e) How could the researchers strengthen their evidence that the effects of DOX on tumour growth in their system are caused by depletion of NF1?
   (6 lines; 3 marks)

f) How does Herceptin suppress a subset of breast cancers?
   (5 lines; 2 marks)

g) How might resistance arise to Herceptin treatment?
   (6 lines; 2 marks)

End of Exam