total 100 points; 1h 45min time; you should ask if a question is unclear; there is sufficient space for your answers, if you need more space your answer is most likely too long.

Question 1: Cancer Stem Cells *(20 points)*

You have the hypothesis that therapy escape of colon cancers after chemotherapy *in vivo* may involve colon cancer stem cells which survive therapy and facilitate relapse.

Design and explain mouse experiments using only keywords which allow you to analyze escape from chemotherapy in vivo: the experiments should enable you to distinguish if surviving cancer cells directly at the end of therapy (all) have CSC properties (consider that only expression of a single marker may not be sufficient), whether the exact phenotype of these cells was pre-existing before therapy or is induced by therapy and further that these cells are indeed causal for relapse. Such experiments will require specific tools. Below you find a list of candidate tools (not all are useful), please **mark the tools** you want to use (you can use additional tools if you think this is required): (**5 points**)

1. an intravital microscope which allows to visualize fluorescently labelled tumor cells in vivo in a mouse (since the mouse survives, repeated measurements are possible)
2. a FACS which allows to quantify fluorescent cells from a tumor
3. a promoter of a known CSC marker which is only active in the CSC population
4. a promoter of a marker expressed in tumor cells but not expressed in CSCs
5. a promoter active in all cells
6. antibodies which can recognize specific markers for CSCs or nonCSCs
7. antibodies to recognize the cell cycle or cell death status (e.g. cleaved caspase 3) of a cell
8. BrdU injection and an anti-BrdU antibody for staining in FACS or histology
9. luciferase cDNAs for the expression of different luciferases with different substrate requirements which produce different colours of light
10. cDNAs encoding fluorescent proteins with different colours
11. a cDNA encoding a fluorescent protein which is stabilized only during S phase so that cell cycle kinetics can be observed
12. a cDNA encodin tamoxifen inducible cre (creERT2)
13. a cDNA encoding light inducible cre (CIP-cre) where cre activity can be induced locally by a blue laser in the microscope
14. cDNA encoding genes which allow elimination of expressing cells (e.g. active caspase 3, HSV thymidine kinase (plus gancyclovir), diphtheria-toxin receptor (HB-EGF) plus diphtheria toxin)
15. a loxP-stop signal-loxP cassette which allows to prevent transcription of a 3' DNA segment unless the stop signal is removed by cre activity
16. DNA sequencing of tumor cells to identify clonal mutations
17. cDNA sequencing of tumor cells to identify expression patterns

analyze if surviving (just after therapy) cancer cells were pre-existing as CSCs before therapy and whether these two populations have identical phenotypes: (**9 points**)

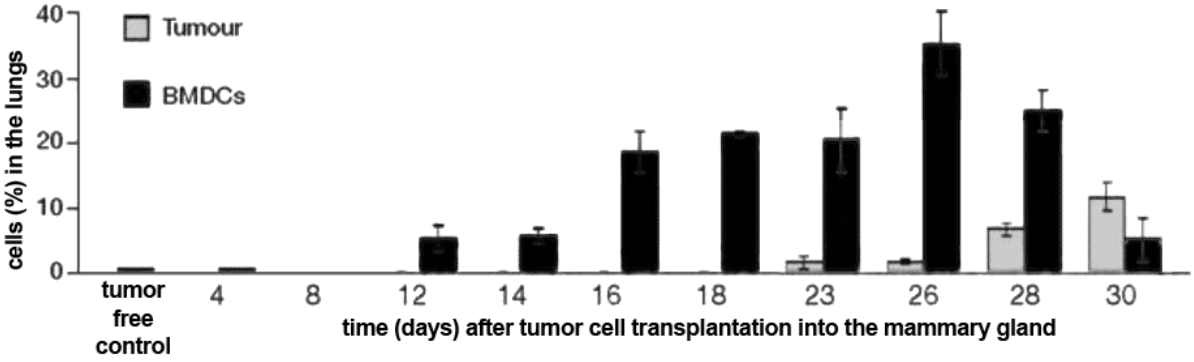
analyze if CSCs, which have survived therapy, are causal for relapse (don't forget that they may be required to establish a tumor in the first place): (**6 points**)

Question 2: Metastasis *(20 points)*

a) Sentinel lymph node analysis has been established in the clinic since some years for the management of breast cancer surgery. It however turned out that the prognostic value of this approach with respect to distant metastases free survival is rather limited.

Based on what you have learned in the course, what could be the reasons for this? Explain your hypothesis and mention evidence that support your concept: **(10 points)**

b) To study the onset of pulmonary metastasis from breast cancers, you observe the lung tissue in a mouse model of orthotopically growing breast tumors in a time-course experiment. You find the following cell frequencies for tumor and bone marrow derived cells (BMDCs) in the lung over time.



How do you interpret these data and what may this indicate regarding a potential role of BMDCs for metastasis initiation? (**5 points**)

Design a simple experiment which would allow you to test for the role of BMDCs? (**5 points**)

Question 3: Cancer signalling (20 *points*)

a) Most signalling pathways use negative feedback loops to terminate pathway activation. For each of the following pathways list one direct target of pathway activation which can inhibit signalling and explain its mode of action: (**9 points**)

Hh pathway regulated inhibitor:

mode of action of the inhibitor:

Wnt pathway regulated inhibitor:

mode of action of the inhibitor:

NFkB pathway regulated inhibitor:

mode of action of the inhibitor:

TGFβ pathway regulated inhibitor:

mode of action of the inhibitor:

BMP pathway regulated inhibitor:

mode of action of the inhibitor:

Notch pathway regulated inhibitor:

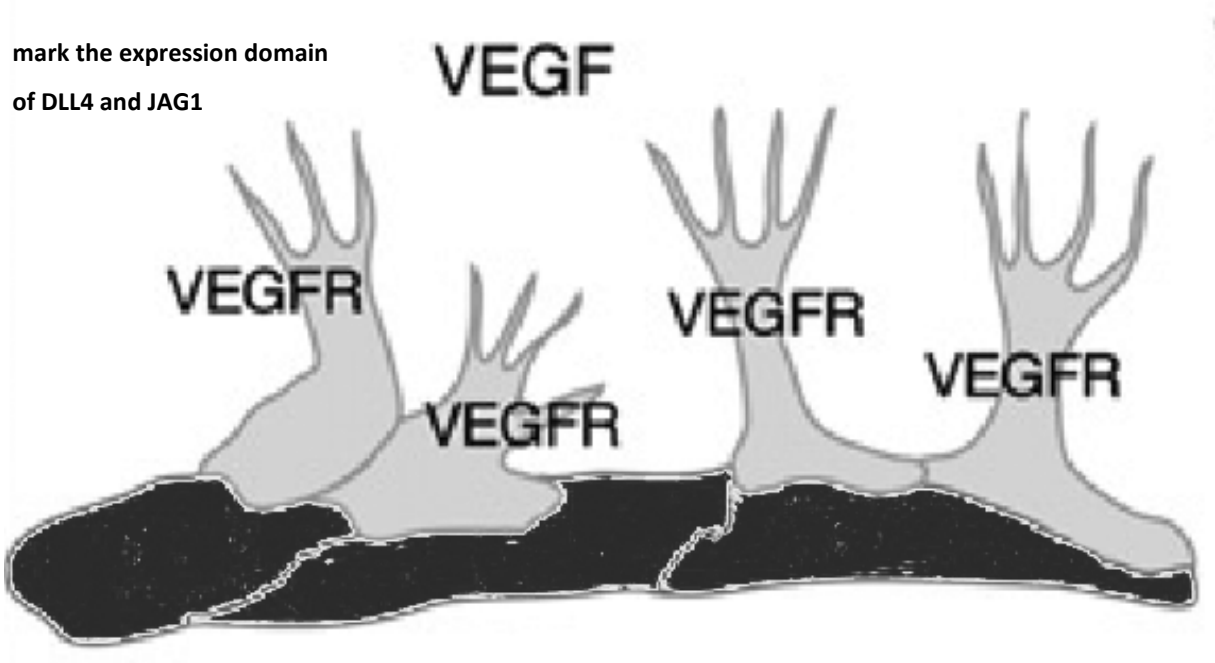
mode of action of the inhibitor:

b) In the course, we had seen how the Notch signaling pathway controls sprouting angiogenesis. In the example we discussed, the following scheme was published which described the role of three components involved in this angiogenic process. Explain the role of VEGF/VEGFR (already depicted in the scheme), name the cells involved, mark in the scheme which cells express Delta-like 4 (DLL4) and Jagged1 (JAG1) (both were removed from the scheme) and explain the role of DLL4 and JAG1 in Notch signalling and how their distribution contributes to the angiogenic process. (**11 points**)

role of VEGF/VEGFR:

cell names: (dark cells) _____ (light cells) _____

mark the expression domain of DLL4 and JAG1



role of DLL4 and JAG1:

Question 4: Cell death (*20 points*)

a) Upon drug treatment, some of the cancer cells you are studying undergo cell death. Based on earlier results you consider ferroptosis or necroptosis as a potential mode of death. How can you distinguish these two forms of cell death by **biochemical assays** and **functional assays** ? (**10 points**)

Ferroptosis, biochemical assays:

Ferroptosis, functional assays:

Necroptosis, biochemical assays:

Necroptosis, functional assays:

b) Cells use endogenous inhibitors to dampen the apoptotic response. Provide two inhibitors of caspases which are involved in the intrinsic and extrinsic apoptosis pathway and explain what caspases they target. **(4 points)**

1) intrinsic apoptosis pathway:

inhibitor: targeted caspase:

2) extrinsic apoptosis pathway:

inhibitor: targeted caspase:

c) Pore-forming proteins play an important role in many forms of regulated cell death. Provide three examples of such pore-forming proteins and briefly summarize their mechanism of regulation. **(6 points)**

1) pore-forming protein:

regulation: ----------

2) pore-forming protein:

regulation:

3) pore-forming protein:

regulation:

Question 5: Histology *and cancer pathology (20 points)*

a) Complete the following sentences and name an example gland for this type of secretion: ***(3 points)***

1) secretion releases secretory products by exocytosis

example gland:

2) secretion loses part of the cytoplasm along with the secretory products

example gland:

3) secretion loads cells with secretory products before they burst apart

example gland:

b) What are the 4 main sub-types of breast cancer and by which markers can these be identified?
mark the subtype with the worse prognosis (5 year survival rate) (**5 points**)

The following page shows H&E histology sections. Please name the highlighted structures:

c) yellow area: _____

blue area: _____

red area: _____

green area: _____

Name the two organs where the yellow/blue and the red/green areas belong to

y/b: _____

r/g: _____

d) blue area: _____

red area: _____

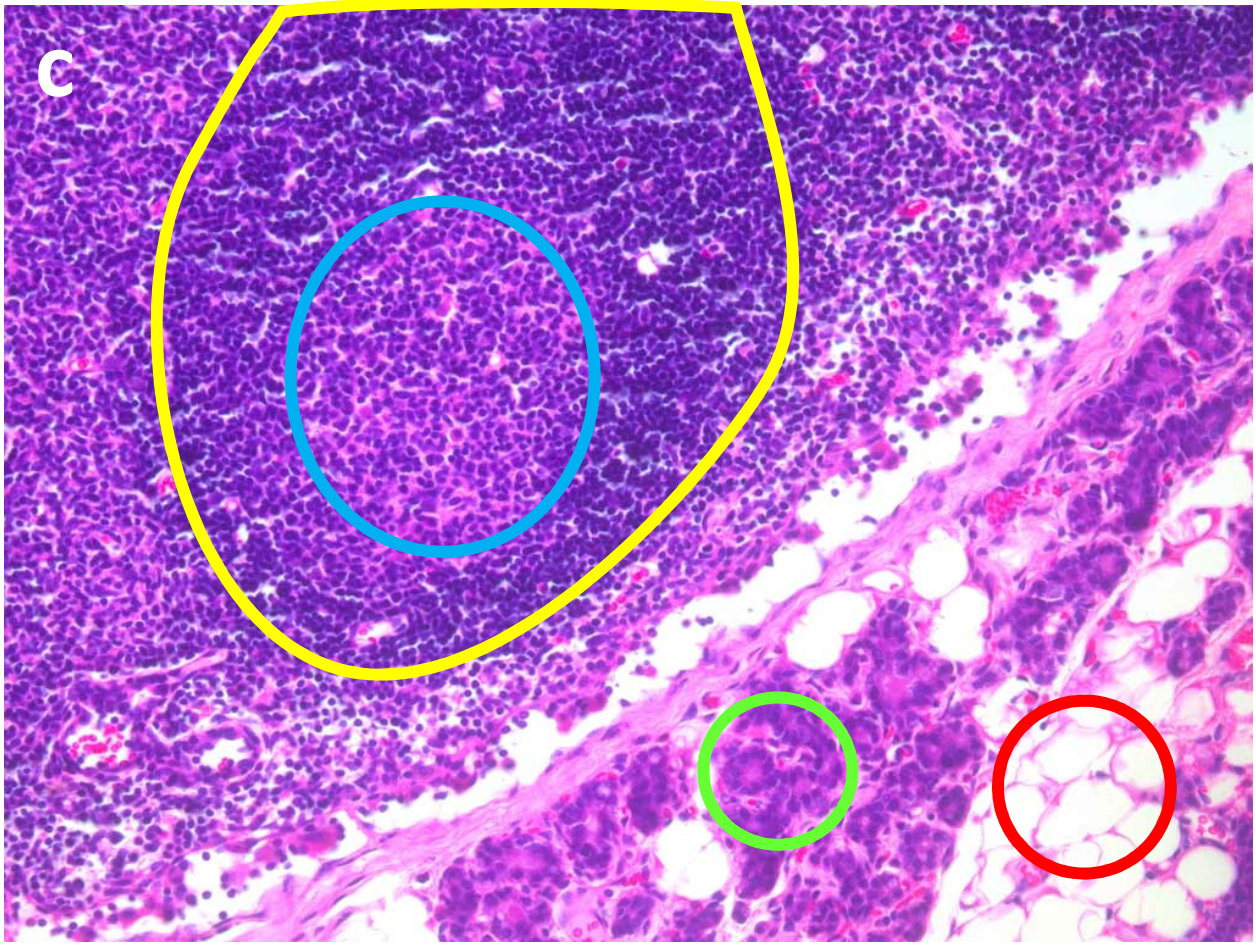
yellow area: _____

e) Grading of tumors relies on a number of criteria, one being nuclear pleomorphism. Which **<u>3 parameters</u>** are scored when nuclear pleomorphism is evaluated

1:

2:

3: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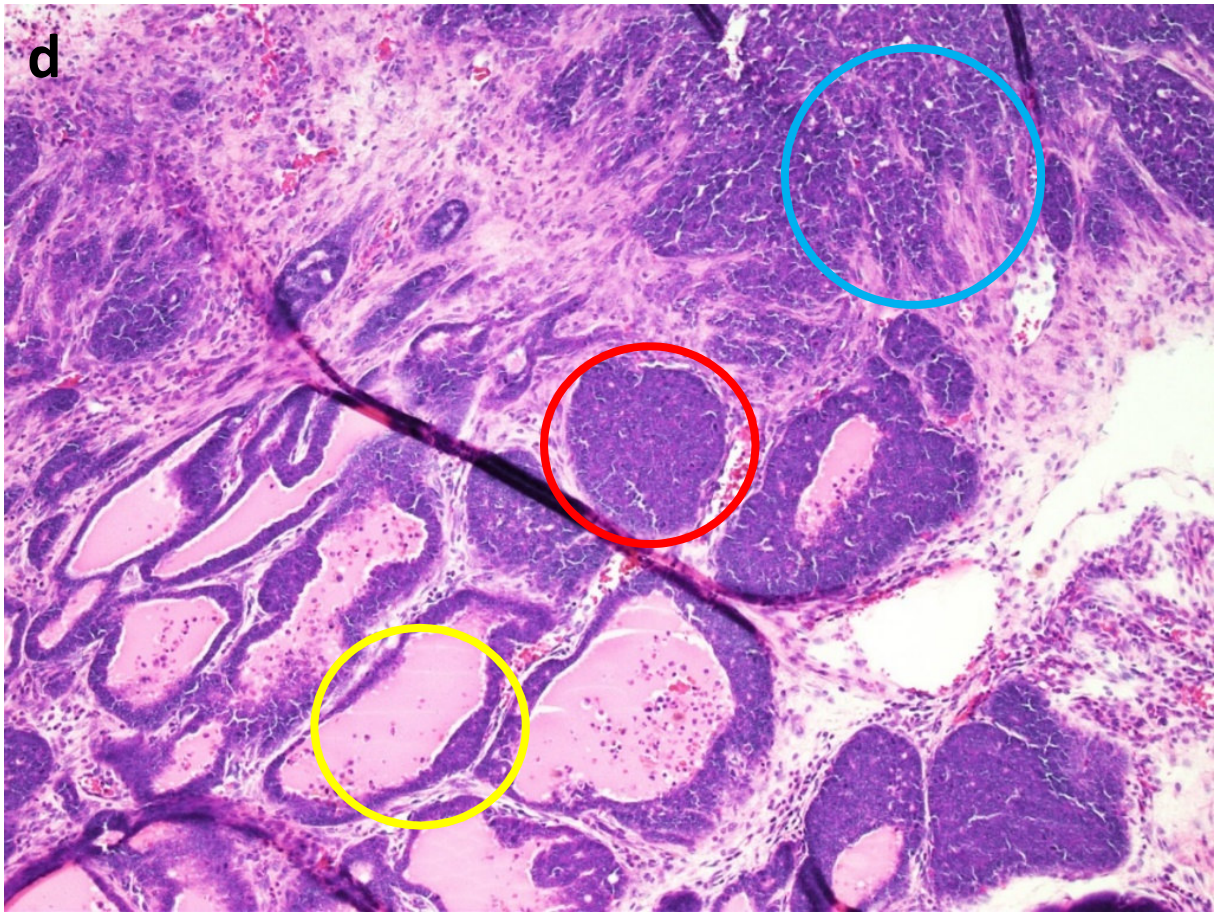
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