Q&A

I have some questions regarding this week paper : https://www.pnas.org/doi/pdf/10.1073/pnas.0707270105

1.Table 1 : I don't understand how to interpret that H1666 EGFR wt and KRAS wt has a IC50 = 4, so is the wild type resistant to Gefitinib ?

Gefitinib is designed to target specific EGFR mutations .
Thus, it can be effective both on EGFR-mutated and wild-type cells.
In this case, it seems that EGFR cannot be phosphorylated and activated,
This might explain partial sensitivity to the drug

Table 1. Summary of phospho-tyrosine profiling of 7 NSCLC cell lines

measured the sensitivity to the EGFR inhibitor gefitinib of seven NSCLC cell lines with different EGFR and KRAS mutational status

Cell line	Mutation status of EGFR, KRAS	Gefitinib sensitivity (IC_{50}, μM)	Phospho-peptides	Nonredundant phospho-proteins	Nonredundant phospho-peptides	Nonredundant phospho-sites
H358	KRAS: G12V	≈10	222	72	89	84
H1650	EGFR: E746-A750del	>10	321	180	251	220
H1666	EGFR: wt; KRAS: wt	≈4	374	219	303	278
H1734	KRAS: G13C	>10	317	201	270	249
H1975	EGFR: L858R, T790M	>10	371	202	302	275
HCC827	EGFR: E746_A750del	<0.1	762	369	575	508
H3255	EGFR: L858R	<0.1	754	373	547	509

resistant IC50 > 5 uM
sensitiv IC50 < 0.1 uM.

In which pathways are predominantly regulated?

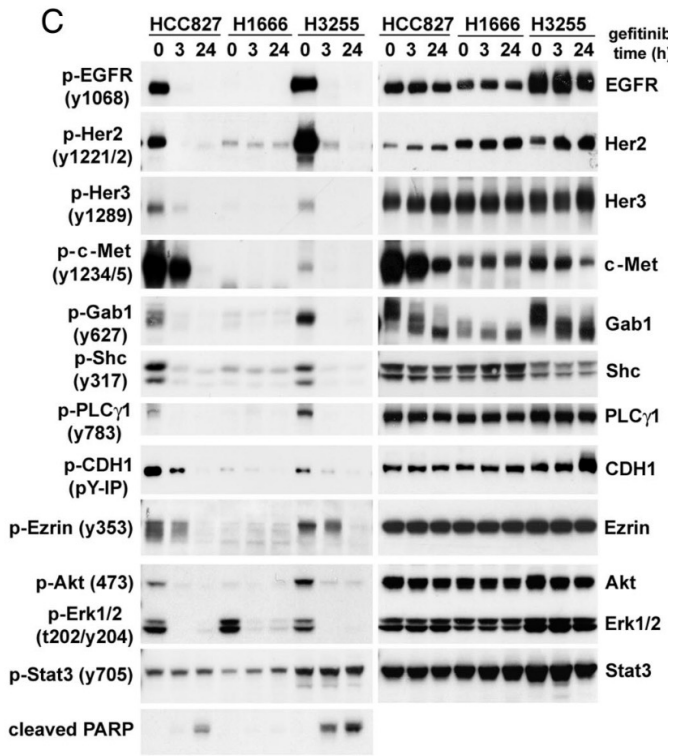


Figure 2 C : How come Stat3 phosphorylation doesn't change upon getifinib treatment, although Stat3 acts downstream of EGFR.

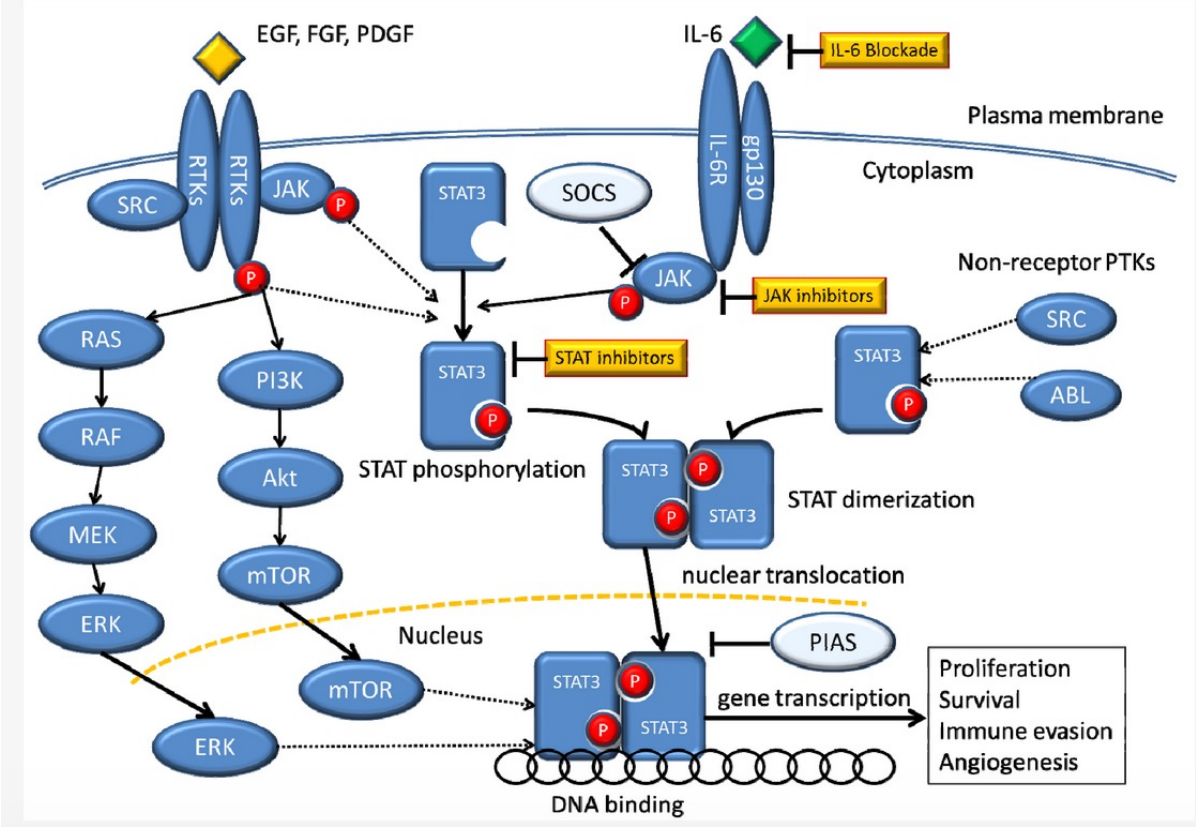
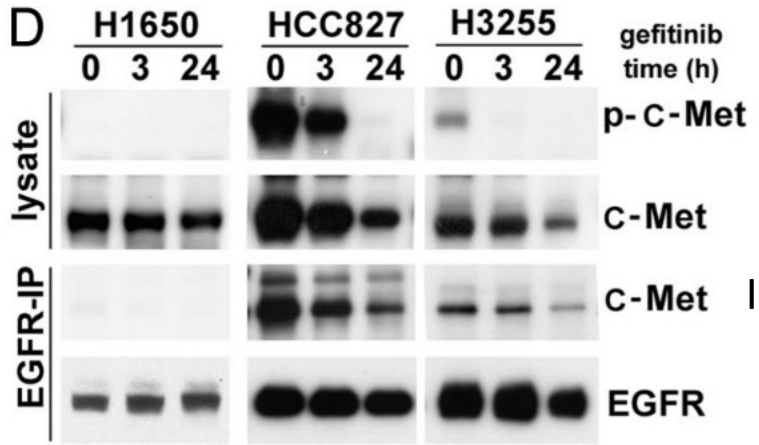


Figure 2 D : Why is c-Met not phosphorylated in the resistant cell line lysate (first line, first column of the blot) ?

It could be mutated, as we don't have info about c-met status in these cells

And why are c-met and EGFR not associated in the resistant cell line (third line, first column) ?



Total lysate

IP

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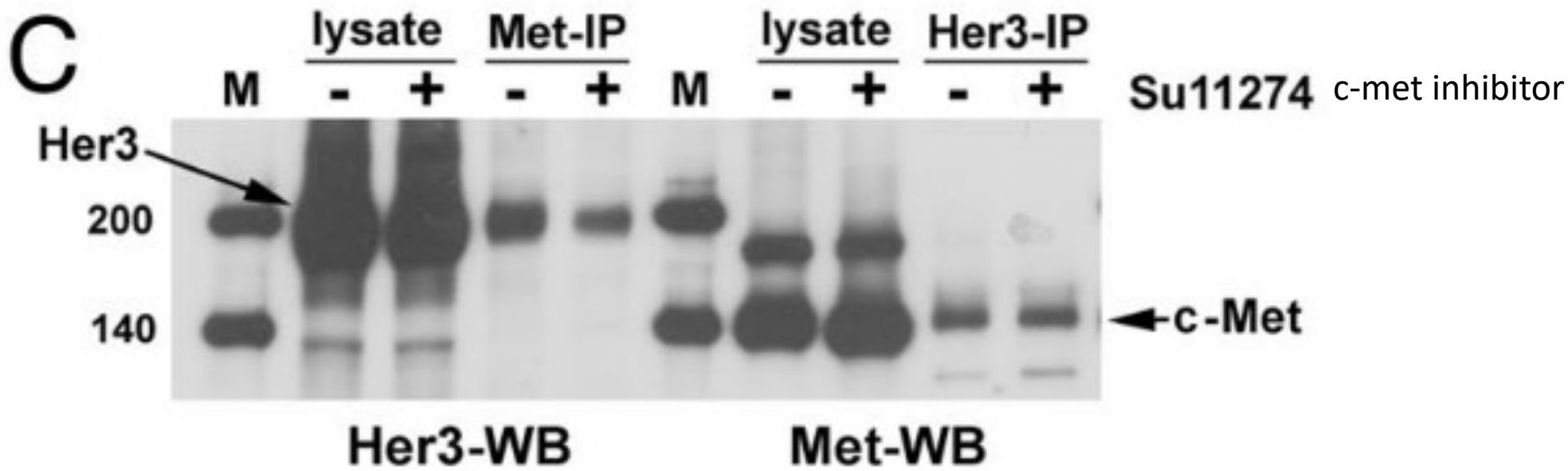
Figure 3 C : I don't really understand how to read this coimunoprecipitation.

This is to understand the co-IP of c-met and Her3 in presence or absence of c-met inhibitor to understand of the inhibitor would affect the formation of this complex.

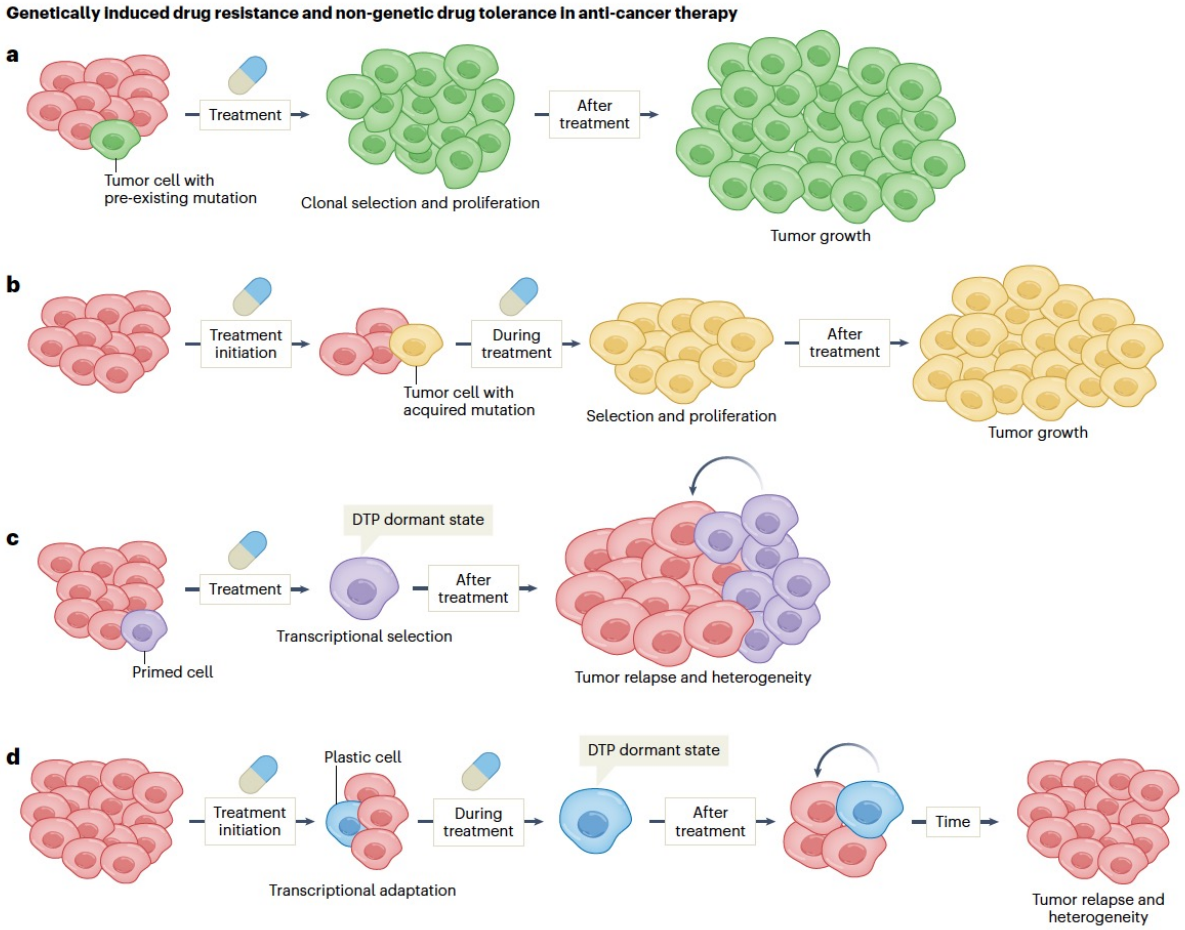


Cancer Cell plasticity can influence tumor evolution and response to cancer treatment

week 10 : last slide, how different can a cancer be after relapse, I understand it can acquire mutations or have transcriptional adaptation, but how far does it go ?

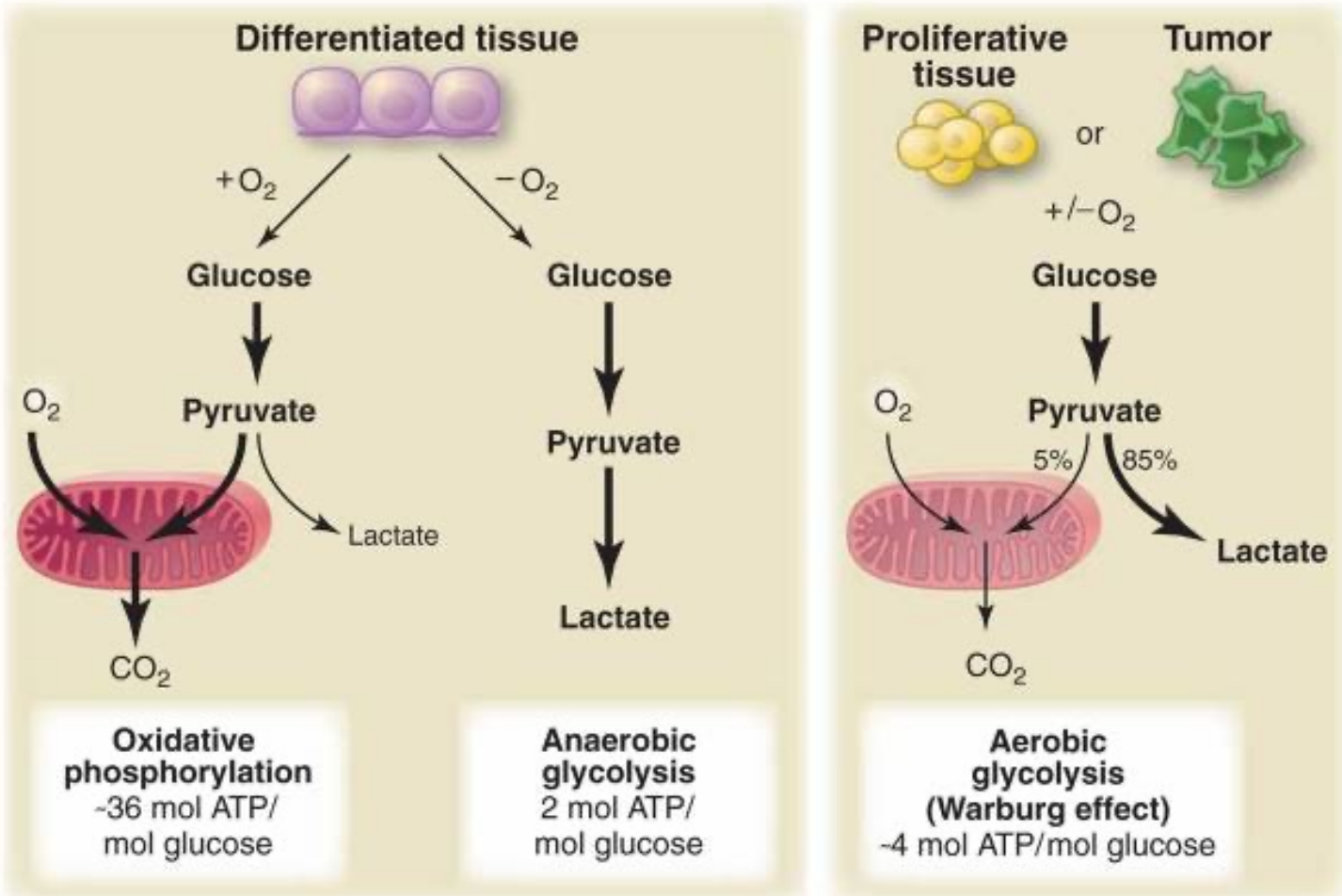
For example, if the patient has a triple negative breast cancer subtype mesenchymal stem like and is first cured but resist and relapse later, can the patient relapse with another subtype of triple negative breast cancer ?

Or more generally, if a patient has a breast cancer HR+ and relapses can it turn into a HR- breast cancer ? How much heterogeneity can be acquired is my question.



week 11 slide 38 : how in depth should we know about the warburg effect ?

If time permits it monday, could you go over it again.
Or is it just relevant that it highjacks synthesis of metabolites useful for cancer cells diet?



Is the part on how we can analyze a pathway’s activity for our understanding of the methods that the paper could apply or should we be able to explain them at the exam ?

Basic understanding of the methods and what can be use for will be enough

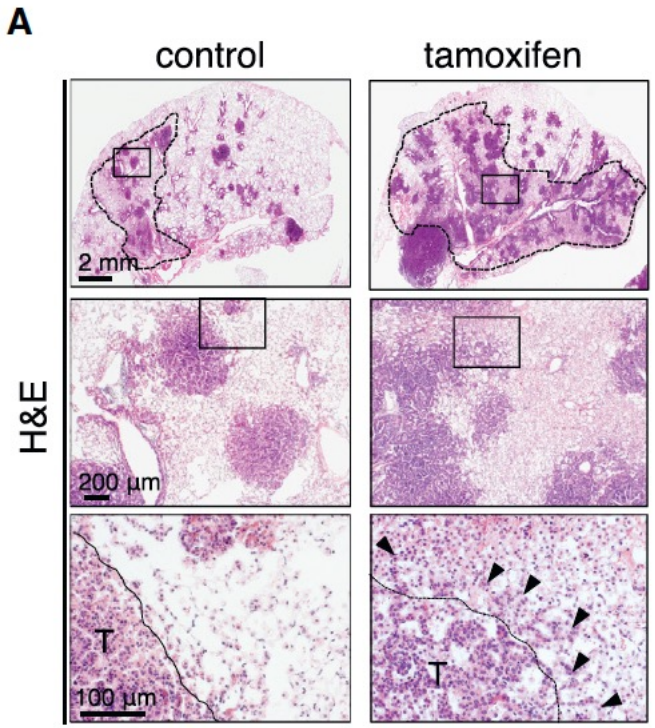
•*They talk about cd31 as a marker for endothelial cells, so if we see it the vessels are mature and if there is no sign of cd31 there is angiogenesis ?*

Usually CD31 should be present, although the expression can be variable between cells

Why do they use this marker first and not directly VEGF to show angiogenesis ?

Highly expressed / good antibody

•For figure 1, the panel A shows H&E staining, how does it work in this case, is it marking the cancer cells ?
And how can it recognize them, is it because of extracellular changes or nuclei changes, like two nuclei indicate mitosis or something else ?



H&E is the combination of two histological stains: hematoxylin and eosin. These are two chemical compounds

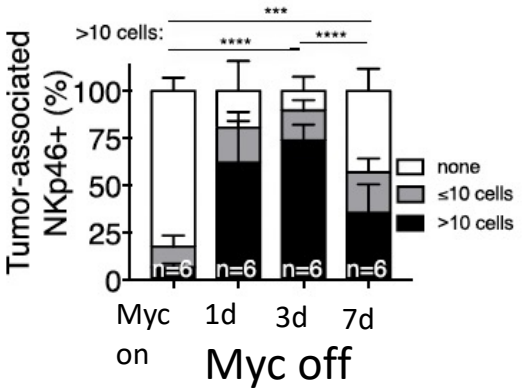
The hematoxylin stains cell nuclei a purplish blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colors.[5][6] Hence a pathologist can easily differentiate between the nuclear and cytoplasmic parts of a cell, and additionally, the overall patterns of coloration from the stain show the general layout and distribution of cells and provides a general overview of a tissue sample's structure.

•For figure 4, we saw in class (attached slide) the mechanism of PDL on T cells but we didn't mention its presence otherwise, here they show that PDL1 is expressed by macrophages, which cells can express PDL-1, is it all immune cells ? or only APC immune cells ? What about NK cells ?

PD-L1 is expressed in tumor cells and in
antigen presenting cells like macrophages, and various other cell type like stroma cells

NK cells express PD1 and engage with PD-L1 on the tumor cells

•Figure 5, in panel A they deactivate Myc and this triggers an influx of NKp46+ NK cells, however why does it decrease again after 7 days ?



Examples of exams questions

1.What is the role of T-reg cells in the immune system and how tumor cells can mimic their activity?

T-reg cells protect the bodies from auto-immune-disease blocking the hyper activation of CD8 T-cells. An equilibrium between CD8 and Treg cells is essential for immune-tolerance.
In tumor microenvironment abundant Treg cells can create an immune-protective environment releasing inhibitory cytokines as TFB-B and IL10.
In melanoma, tumor cells secret TGF –B to mimic the action of Treg cells and block CD8 activation.

2.. Can you briefly summarize how activation of membrane receptor can induce changes in gene expression? BONUS: Provide a graphical representation for a specific example (+2 points).

Autocrine or paracrine molecules induce dimerization of trans-membrane receptors (e.g. EGFR) that will activate membrane bound proteins (RAS or PI3KCA) inducing activation of downstream molecules through conformational changes (BRAF) or phosphorylation (AKT) promoting a signaling cascade (MAPKK, MAPK or mTOR) that will induce nuclear translocation of transcription factors (e.g. MYC) and gene expression changes.

Describe two possible mechanisms of resistance to immunotherapies and their functional effects.

B2M deletions or mutation (loss of MHC class I expression)
Over-expression of TGF-B to block CD8 activation

Ibrutinib is a specific inhibitor of Bruton tyrosin kinase (BTK) approved for the treatment of leukemia patients. Sequencing studies have already identified a hotspot mutation that induces resistance to ibrutinib in a small group of patients. How can you investigate if additional mutations in BTK can promote resistance? Briefly describe the method.

Performing a mutagenesis screen.
1. Clone BTK cDNA in a plasmid
2. Use a genetically modified bacteria strain triple negative for DNA repair to amplify the DNA and randomly generate mutations in BTK coding sequence.
3. The mutagenize library will be used to transduce the leukemic cells.
4. Then, cells will be treated with Ibrutinib.
5. Cells expressing mutated BTK that confers resistance to ibrutinib will be positively selected.
6. Sequencing analysis of the resistant population allows the identification of specific mutations associated with resistance.