IBMS4 ICA2

Research Programme Deconstruction

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Word Count: 1480

Introduction

Drug resistance is a major obstacle for effective cancer therapies. Chemo-drug dosage limits and pharmacokinetics, genetic mutation, environmental protection and drug-resistant cell formation and accumulation contribute to cancer drug resistance (Housman et al., 2014; Vasan et al., 2019). Cancer stem cells (CSC), or an emerging concept linking cancer progression to a small group of cancer-initiating cells (Figure 2), are associated with drug-resistant cancer cell accumulation (Figure 1A). Thus, the research vision is reasoned, i.e., **eliminating drug resistance by targeting CSC** (Zhang et al., 2017). To achieve this, three questions remain to be answered (Figure 1B):

1) whether CSC is responsible for drug resistance in specific cancers,
2) how CSC is regulated, and
3) how CSC can be used as a therapeutic target.

In the seminar, Dr Wei Guo presented two studies, both related to NOTCH-mediated CSC growth manipulation as a therapeutic option: one published in 2017 (Zhang et al., 2017), and another not yet published. This essay aims to review the studies in the seminar using the **research deconstruction framework**. Thus, we will appreciate the research question construction, the methods to address the questions and conclusions drawn from reasoning about data in the two studies.

Research approaches

Research I: from mechanistic research to therapeutic application

- **Sub-questions and methodology**

Research I focuses on lung cancer, the top malignancy in China. The research data are published, clearly labelled, and accessible online. Through the **collaboration** with clinicians, the author studies CSC in lung cancer (Figure 3A), with cutting-edge techniques, e.g. the patient-derived sphere-forming assay (PD-SFA) and orthotopic xenograft assays (OXA) for clinical lung cancer samples developed by the authors, in addition to traditional molecular biology approaches, e.g. western blot, which together properly addresses most questions.

To understand **whether CSC is responsible for drug resistance in lung cancer**, the authors hypothesise lung CSC (LCSC) to be a major source of resistant cancer cells. Then, how we can study LCSC is our next question. FACS can effectively sort cells based on surface markers and molecular characteristics. Thus, **identifying suitable markers** are crucial. **How can we examine the markers?** The authors use PD-SFA to check in vitro whether the sorted cells are self-renewable as expected.

With the markers identified, **whether CSC can initiate tumour growth and drug resistance** should be examined. The authors use PD-SFA and OXA to examine the renewability to check **whether CSC can initiate cancer**. By using cisplatin in OXA and taxol in PD-SFA, the authors check **if chemotherapy causes LCSC accumulation, which suggests cancer drug resistance,** in vivo and in vitro, for cisplatin and taxol and two common chemo-drugs.

With the drug-resistant LCSC, **what cause drug resistance of CSC** interests researchers. In particular, **NOTCH signalling** that regulates normal stem cell development and differentiation could be responsible, although **other signalling pathways may be involved**. The speaker's research paper suggests they manipulate NOTCH1 expression with shRNA to explore the impact on NOTCH signalling so that they can postulate potential signalling pathways accordingly and then tested **whether pharmaceutical manipulation of NOTCH pathway with an existing compound can restore drug sensitivity** to further support the pathway and possible therapeutic application.

- **Reasoning about data**

In FACS, $CD166^{+}CD49f^{hi}CD104^{-}Lin^{-}$ is found to be associated with tumorigenic and self-renewable LCSC, as confirmed by PD-SFA, while drug resistance is confirmed with cisplatin and taxol added in PD-SFA and OXA. Thus, LCSC accumulation suggests increased resistant cells, which is further confirmed in clinical patients. Yet, note that using different drugs in different experiments (i.e., PD-SFA+taxol & OXA+cisplatin) **may not be strict controls of each other**, considering the drugs' different effects (Postmus, 1999).

The data indicating NOTCH's role is not presented, yet in the paper, it is being discussed detailedly (Zhang et al., 2017). γ-secretase inhibitor impacts NOTCH1 function in a dose-dependent manner, where high dose causes HES1-mediated inhibition of LCSC cell renewable and low dose causes platinum resistance (Figure 4). Aiming to support the potential therapeutic application, two PD-SEA reveal that the combination of γ-secretase

inhibitor and chemo-drug eliminates most LCSC. **Yet**, the efficacy of the drug combination greatly varies in two samples (Figure 5), suggesting patient heterogeneity or other confounders worth investigating, in addition to further clinical trials.

In conclusion, the research supports a NOTCH1-dependent LCSC growth that confers drug resistance, whereas NOTCH1 inhibition eliminates resistant cells when combined with chemotherapy. The sub-questions are closely linked and supplementary to each other, which together support the conclusion (Figure 3A).

Research II: from therapeutic application to mechanistic research

- **Sub-questions and methodology**

Research II introduces anti-proliferative agent 389-3 and its derivative 363-3 and studies the mechanisms underlying their anti-proliferative function (Figure 3B). The methodology is not described detailedly due to the limited time, yet the presented data mostly suggest traditional methods. The data for the research is unpublished and incomplete, due to ongoing research.

To understand the drug effect, we need to know **what the drug targets are.** According to the seminar, 389-3 is found to target translational machinery via eIF4a. G-quadruplex (GQ) confers eIF4a-dependent translation, which means 389-3 should inhibit the translation of GQ-containing DNA. Thus, the drug-target interaction can be confirmed with GQ-c-myc depleted by 389-3 compared to random-sequence c-myc in western blot, as eIF4a blockade via 389-3 blocks oncogene translation (Jin et al., 2013; Wolfe et al., 2014).

Then, we need to know **how 389-3 arrests tumour growth**. 389-3 and CR-31-B are both eIF4a-dependent anti-proliferate agents. eIF4a-dependent translation regulates oncogene expression regarding multiple cellular functions, especially proliferation, where c-myc is a key player (Chan et al., 2019). Thus, the authors examine **whether they both interact with c-myc**, which is tested with the sphere-forming assay (SFA) in vitro.

Also, given the various functions related to eIF4a (Figure 6), it is interesting to know **how 389-3 responds to different cell types**. The authors study 389-3 in CSC with PD-SEA in vitro and an ER2 mouse model in vivo. Then, as summarised in the seminar, eIF4A-

dependent translation is linked to proliferation, which involves NOTCH1. Thus, it is worthwhile to know **whether NOTCH1 is associated with 389-3's anti-proliferative effect**. 389-3 and CR-31-B may deplete NOTCH1, which is tested with SFAs.

- **Reasoning about data**

Reduced survival of various cancer cells and suppressed tumour growth demonstrates the anti-proliferative effects of 389-3 in vitro and 363-1 in vivo, respectively. However, the similarities or differences between 363-1 and 389-3 are not reasoned with more data.

According to the western blot data, 389-3 is sensitive to GQ c-myc, whereas random c-myc is not impacted by 389-3 addition. Thus, it is likely that **GQ translation is halted with 389-3, yet this does not directly indicate the interaction with eIF4a**. Further cell viability studies show that both 389-3 and CR-31-B-treated cells can be rescued by non-GQ c-myc, suggesting GQ-c-myc inhibited by 389-3 or CR-31-B. Thus, **c-myc is required for the antitumour effect**, yet it is not sure whether GQ is also required.

SFAs show only **389-3 eliminate CSC, while CR-31-B don't**. OXA further tests the result in vivo, which turns out **389-3 effectively eliminate nearly all cancer cells, in addition to CSC, while CR-31-B treatment leads to residual cells,** in the mouse ER2 cancer model. Cell sorting results show that more than 90% of CSC, as marked by $CD49f^{hi}CD61^{+}$, is eliminated by 363-1. NICD is the intracellular domain of NOTCH1, which can rescue NOTCH1 depletion. SFAs show 389-3-treated cells are significantly less than the untreated cells, yet those treated with NICD almost equal to untreated cells. **Although the data support NOTCH1 anti-proliferative effect, whether NICD can directly rescue cancer cells after 389-3 treatment is unclear.**

In conclusion, the research supports that 389-3 inhibits CSC proliferation by targeting eIF4a-dependent translation, which is associated with NOTCH signalling downregulation (Figure 3B). The sub-questions in the research are mostly discrete, and the data is not solid enough to go against alternative hypotheses/models. For example, it is unclear whether the residual cells of CR-31-B treatment are CSC or other cells. Thus, more research is needed to exclude alternative possibilities.

Future directions

Overall, the two studies support the potential of cancer therapies targeting CSC. Yet, for research question construction, **alternative hypotheses should be examined, with** both positive and negative controls to be strictly defined. Therefore, signalling pathways beyond NOTCH should be explored (Figure 3), **to address potential stem cell–niche signal shifts during cancer progression** (Batlle & Clevers, 2017). **Besides, EMT that is mentioned but not discussed in the seminar, should be further researched**, regarding its contribution to CSC drug resistance (Singh & Settleman, 2010; Batlle & Clevers, 2017) For reasoning about data, **statistical approaches, e.g., power analyses, significant level, should be clarified.** Not all sample sizes are labelled (e.g. Figure 7C). Uneven grouping (e.g. Figure 7A) and small sample size (e.g. Figure 7B) could skew the conclusions. For potential therapeutic application, the authors need to further research on **the proper time and method for intervention** (Figure 8) and **the markers to identify proper patients** due to patient heterogeneity (Figure 5; Clarke *et al.*, 2006).

Figures

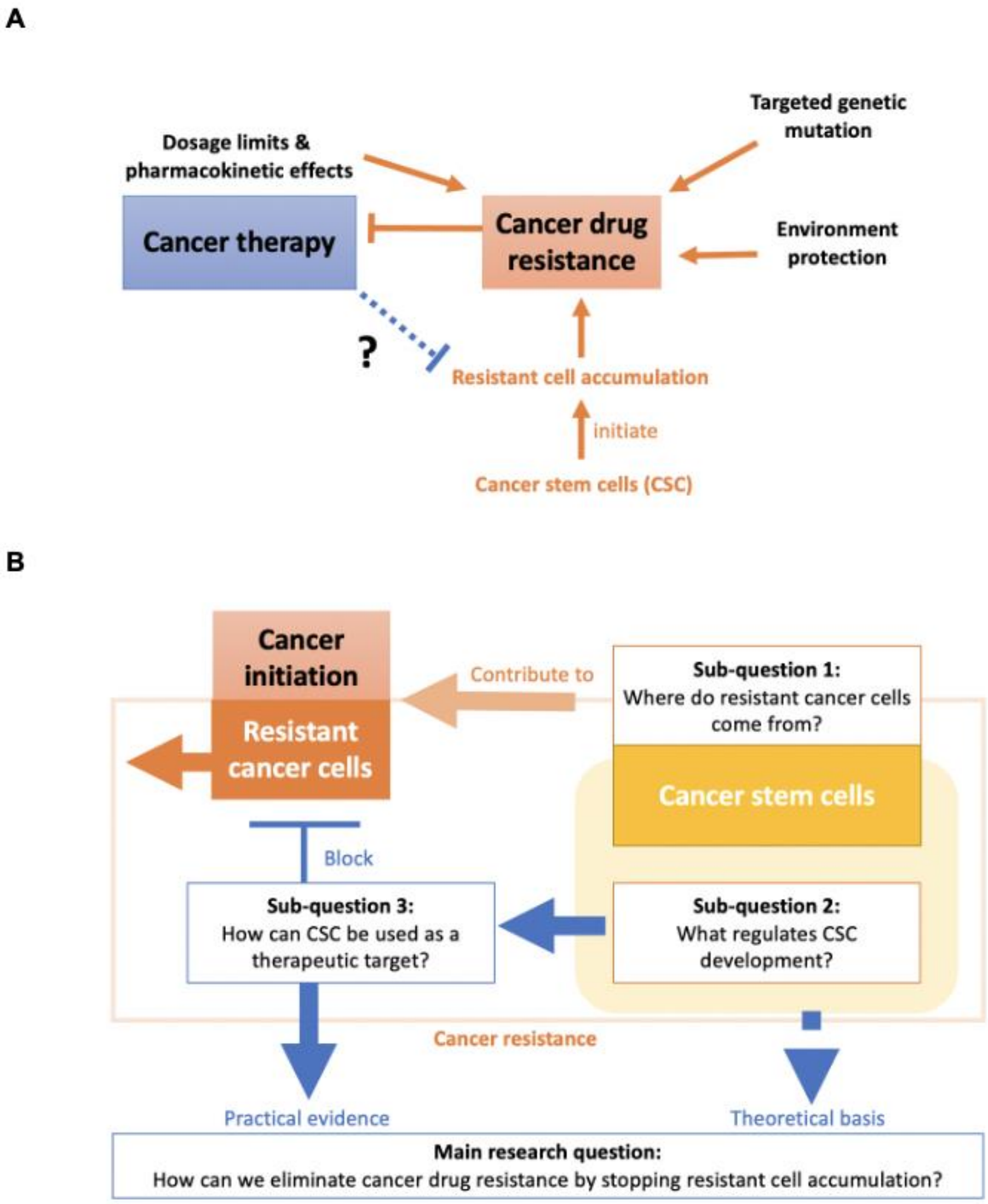


Figure 1. Research question deconstruction.

1A | The big picture question: how can we stop resistant cell accumulation to eliminate cancer drug resistance?

1B | Sub-questions: 1) where do resistant cancer cells come from? 2) what regulates CSC development? 3) how can CSC be used as therapeutic targets?

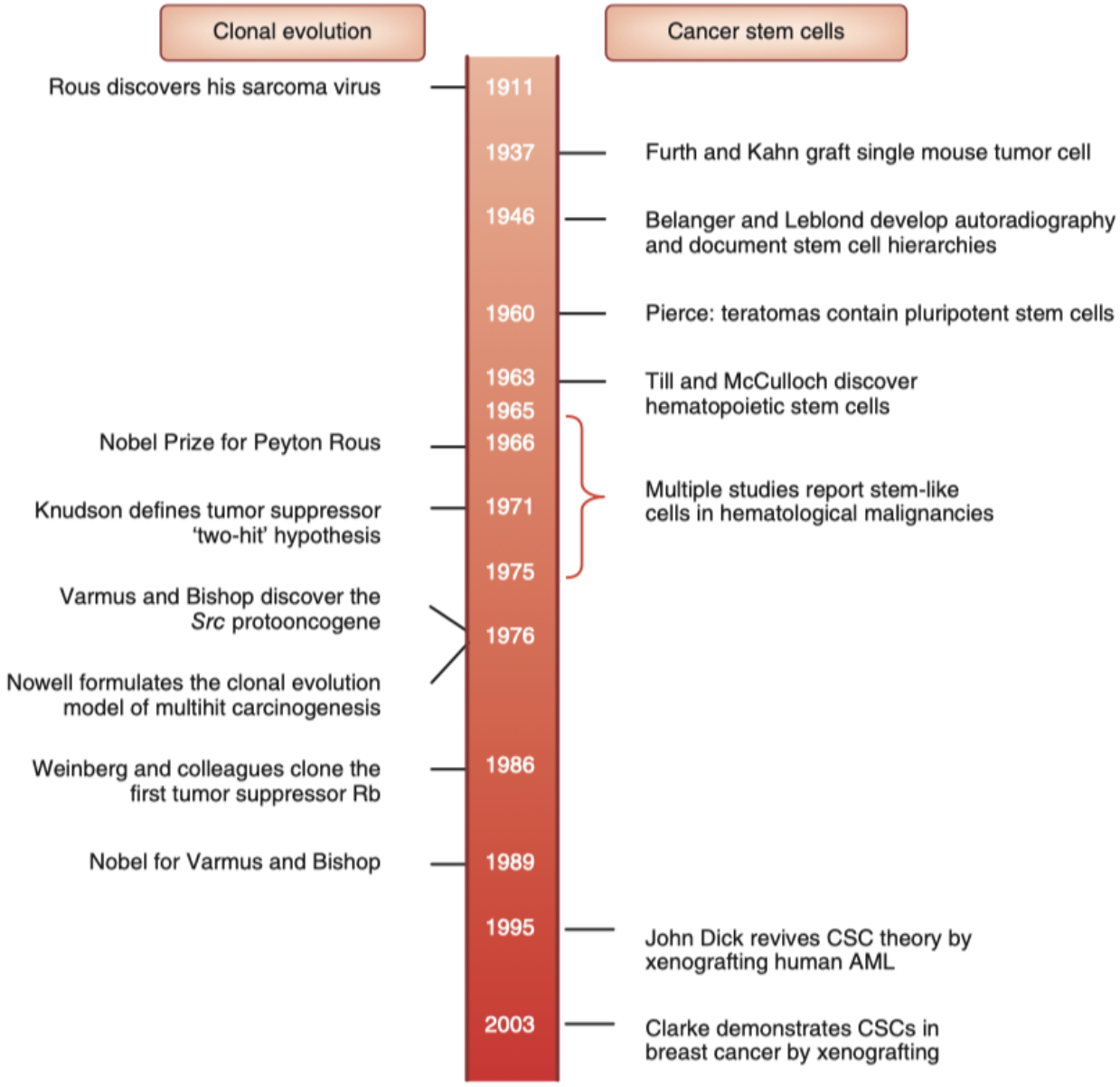
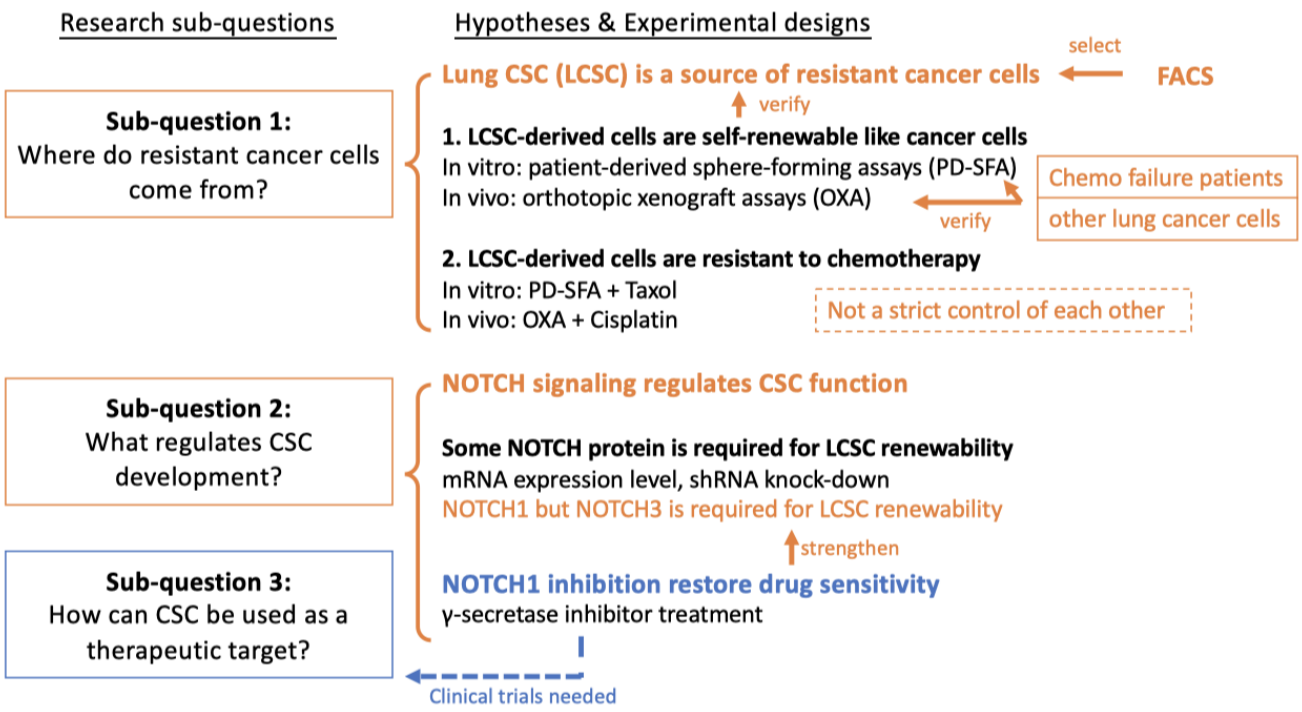
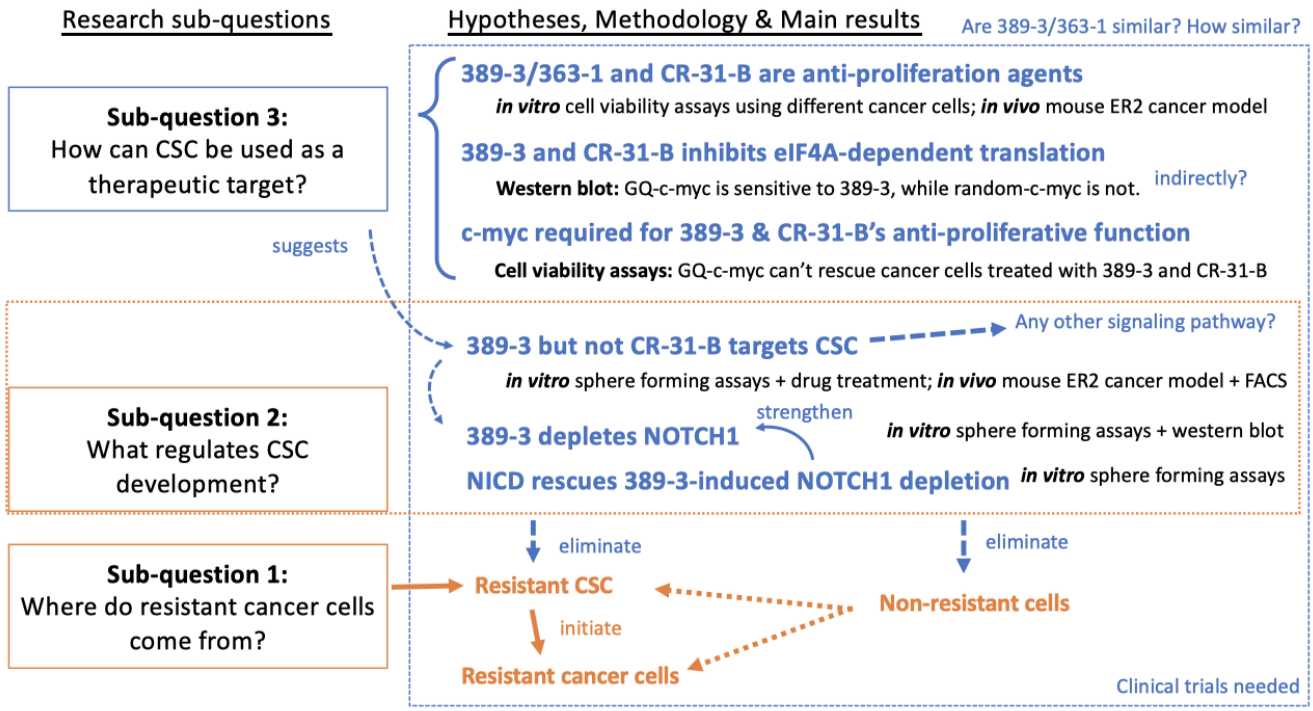


Figure 2. Milestones in CSC research. (Clevers, 2011)



Solution 1: combination of tumour resensitiser and traditional chemo-drug

B



Solution 2: a single drug to eliminate both sensitive & resistant cells

Figure 3. Research approach deconstruction

2A | Research I: from mechanistic research to therapeutic application

2B | Research II: from therapeutic application to mechanistic research

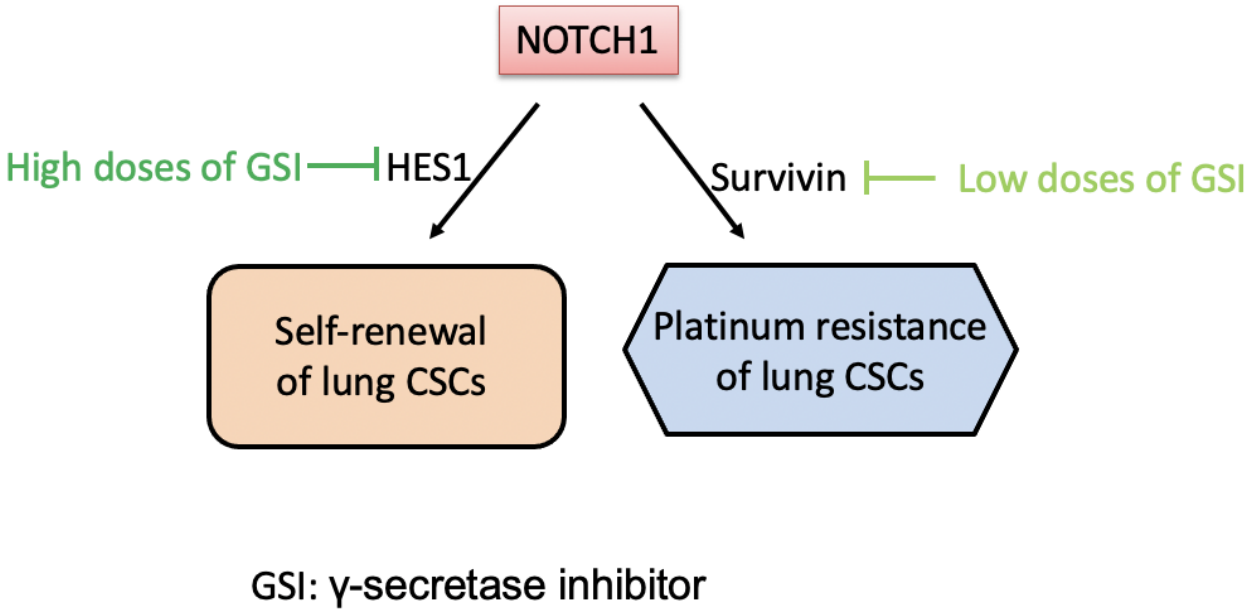


Figure 4. The γ-secretase inhibitor (GSI) impacts NOTCH1 signalling in a dose-dependent manner. (Zhang *et al.*, 2017)

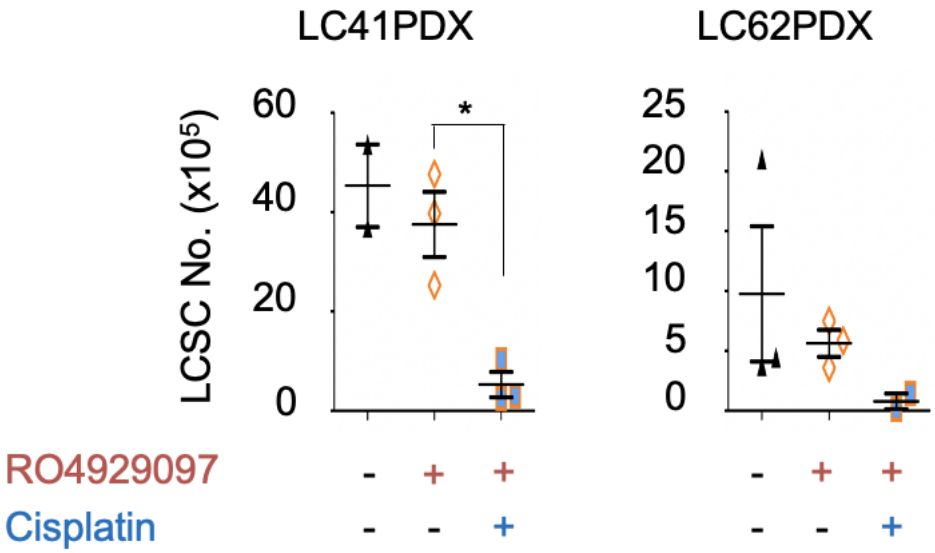


Figure 5. The effect of γ-secretase inhibitor (RO4929097) combined chemo-drug (cisplatin) in two patient-derived xenografts (PDX) of patient LC41 and LC62. (Zhang *et al.*, 2017)

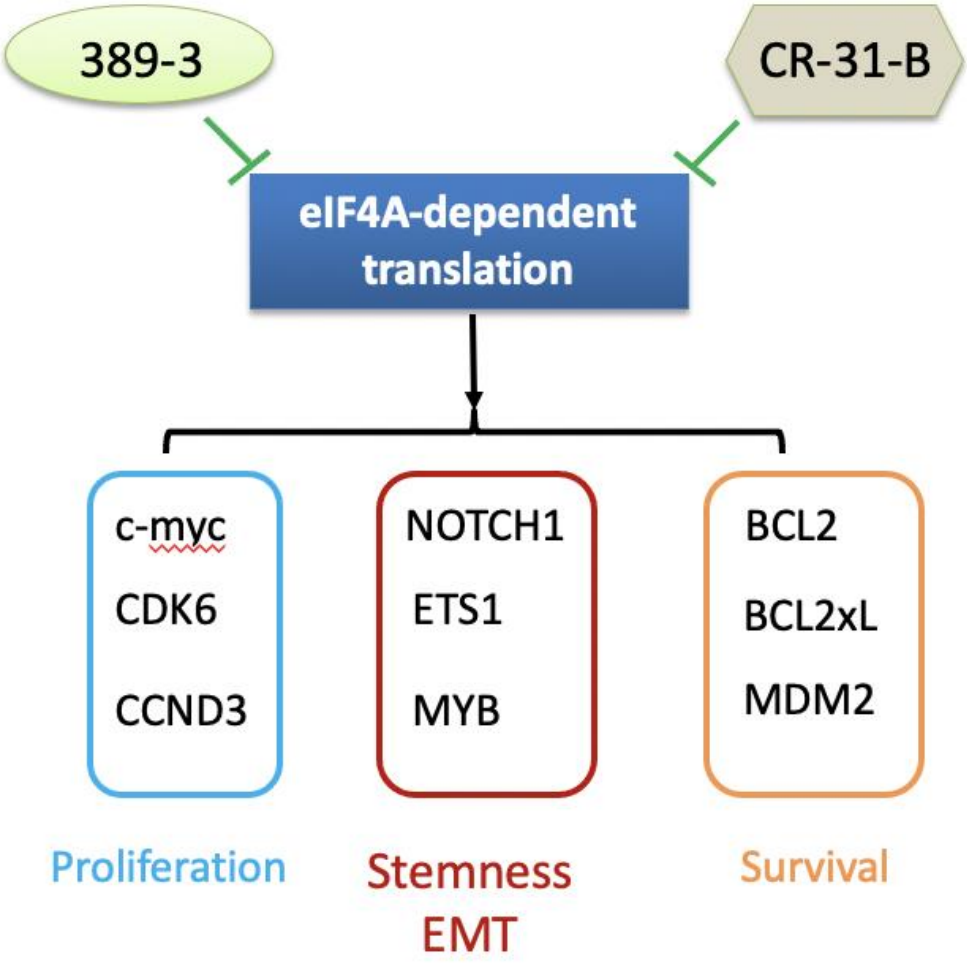
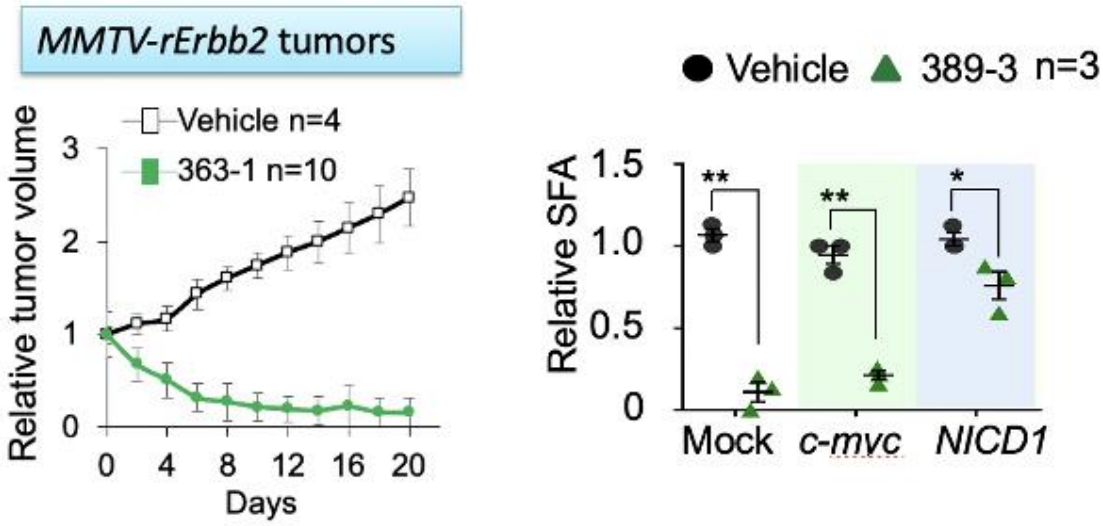
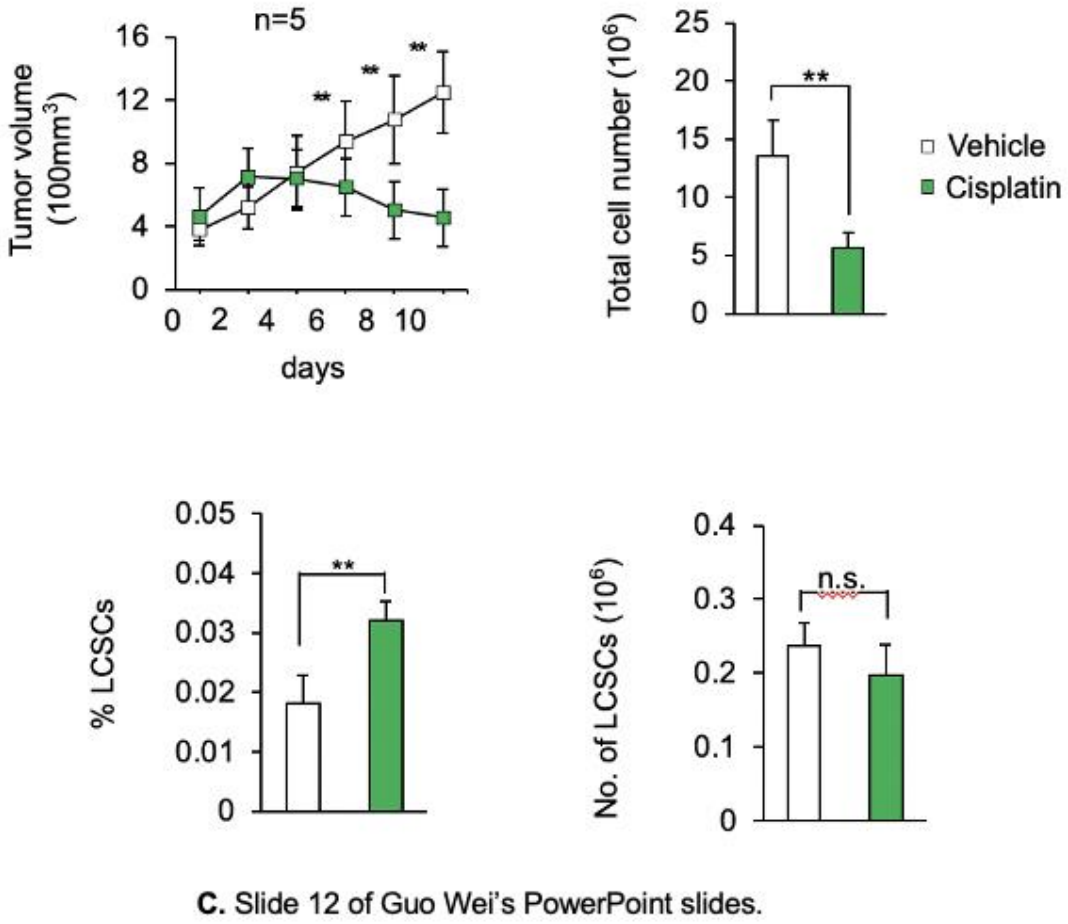


Figure 6. The various function related to eIF4A-dependent translation. (Guo, 2020)



A. Slide 17 of Guo Wei's PowerPoint slides. **B.** Slide 22 of Guo Wei's PowerPoint slides.



C. Slide 12 of Guo Wei's PowerPoint slides.

Figure 7. Example of vague data presentations in the seminar. (Guo, 2020)

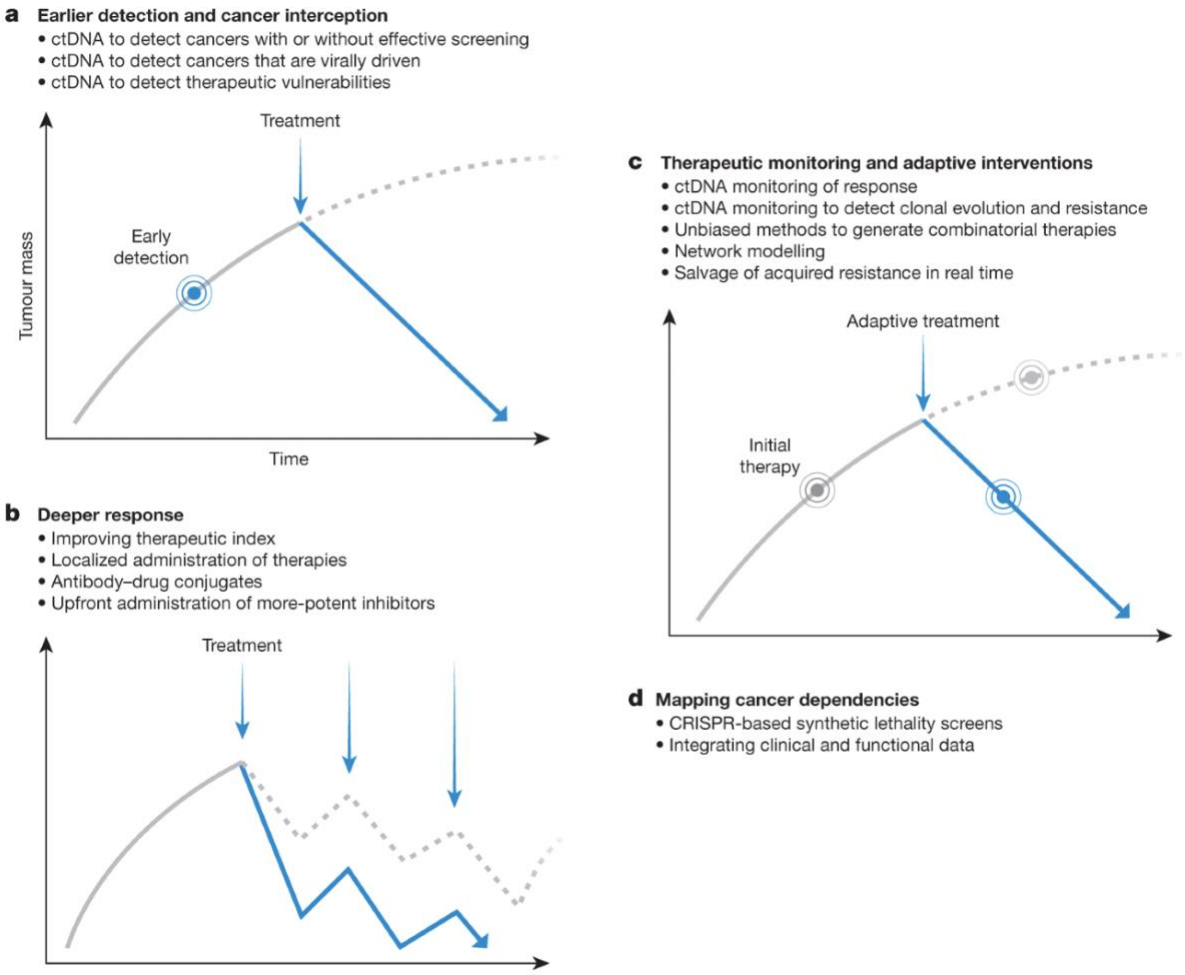


Figure 8. Various methods of clinical intervention to counter drug resistance (Vasan *et al.*, 2019).

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