Non-small cell lung cancer

Valorization

Clinical relevance

Cancer and cardiovascular diseases are the foremost causes of death worldwide. According to the 2018 report from the American Cancer Society, the 5-year relative survival rate for the combination of all cancers is 70 versus 63% in white and black races, respectively. Specifically for lung cancer, the 5-year survival rate is 18% and it can go up to 56% if it is localized, although only 16% of lung cancers are diagnosed at an early stage. For glioblastoma multiforme on the other hand, about 5% survive over 5-years but most patients will survive only for a year. Standard first-line treatment for glioblastoma has been clearly defined since 2005 as a combination of radiotherapy and temozolomide treatment, however, there is still controversy on further lines of treatment. There is a myriad of treatments available to treat cancer, some of which are efficient in initially reducing tumor burden, however, this positive outcome is not always long-lasting and within a few years the cancer recurs. It is this population of resistant cancer stem cells that we have aimed to tackle in this thesis by trying to prolong the effects of current standard of care (chemoradiation) combining it with a resistant cancer stem cell targeting agent: a NOTCH inhibitor.

Monotherapy treatments with NOTCH inhibitors have not been successful in clinical trials and many have been halted. However, this effect should have been expected since NOTCH inhibitors only target a small percentage of cells from the bulk of the tumor, and its effects are likely to be potentiated when in combination with therapies that target a characteristic of all cancer cells such as uncontrolled cell division. Evidence of the latter can be seen in this thesis in preclinical studies both in non-small cell lung cancer (NSCLC) and glioblastoma (GBM). One of the mayor challenges of NOTCH-based inhibition therapy is gastrointestinal adverse effects, resulting in the induction of diarrhea, due to the requirement of NOTCH protein in normal tissues. Nevertheless, in this thesis we have shown that intermittent scheduling, reduced dosing, and its combination with other therapies with which it synergistically interacts, greatly improves tolerability. We are not pioneers in showing that intermittent dosing reduces the side effects of NOTCH inhibitors. Other groups have also shown that using inhibitors that target a single or two NOTCH receptors different from NOTCH1, also reduces toxicity. In this study, we show that using a NOTCH inhibitor (BMS906024) that targets potently and equally all NOTCH receptors, at a low dose and with an intermittent schedule, is tolerable. The use of pan-NOTCH inhibitors would avoid pre-screening of tumors for the specific NOTCH receptor that is overexpressed, and a general NOTCH activity screen would be sufficient. In hematological tumors, the use of glucocorticoids in combination the NOTCH inhibition treatment, attenuates the adverse effects upon the gastrointestinal

tract. Recently, there have been reports of nanoparticles with glucose moieties loaded with γ-secretase inhibitors, that are internalized by breast cancer cells and stem cells in preclinical studies, where the uptake is correlated with the glycolytic profile of the cells and can efficiently reduce the cancer stem cell population within the tumor (Mamaeva, V *et al*. Molec Ther 2016; doi: 10.1038/mt.2016.42). These targeted-delivery strategies could help minimize the adverse effects upon normal tissues.

Societal relevance

Doctors and scientists are continuously on the lookout for new ways to improve treatment, may it be through the accidental discovery of new drugs due to explosions or leaks into the environment; testing of plant, fungi, or animals; comparing the biology of cancer versus healthy cells; or by *in silico* simulation of the interaction between the potential new drug with its target. These new drugs are then tested first *in vitro* and later *in vivo* in two or more animal species, a process that will take ~3-4 years, to evaluate whether the drugs do what they were designed for, and the potential side effects they may have, thus aiding in translation to human clinical trials. In clinical trials, doctors will collect information from 20-80 volunteers for ~1 year, on the dose at which the drug is effective, how it is metabolized, absorbed, distributed, excreted, and at which dose side effects are developed (phase I). In phase II clinical trials, minimum and maximum doses are identified in 100-300 volunteers for the following ~2 years, to assess the drug's effectiveness for a specific cancer. In phase III clinical trials, it will be assessed how well the new drug works compared to standard treatment and the potential adverse effects it may have in 1000-3000 volunteers for ~3 years. At this stage, the Food and Drug Administration (FDA) will review the data available for the drug (~2-3 years) and if the drug is effective, safe, and works better that standard of care, the FDA will grant approval for marketing. However, there is a last phase (phase IV) where long-term effects of the drug are assessed over the course of the years. This means that from the time that he drug is discovered until it is up in the pharmaceutical shelves where it is sold, it takes ~12-14 years and a considerable amount of medical, scientific, and economical (in the order of billions of dollars) resources together with a substantial quantity of patient volunteers. Despite careful designing and preclinical efforts, only about 5/5000 drugs proceed to clinical trials, and 1/5 of the drugs that do proceed, actually make it to the market (www.medicinenet.com).

The cost of health care for cancer patients is increasing not only due to inflation but also due to an increment in the number of procedures, cases, and the ageing of the population. Because cancer therapies, especially those involving stem cell

transplantations and palliative chemotherapy in NSCLC for example, are resource intensive, investors in cancer research (such as pharmaceutical companies and individuals who donate to the cause), and cancer patients who pay for the treatment, want to see the benefit of additional procedures and drugs, thus placing constraints on the expenditures. There is therefore a great need to improve decision-making to improve cost-effectiveness of tested treatments, and suggested treatments must show benefit versus current standard of care treatments. To meet this end, in this thesis we have suggested to use a new triplet combination consisting of standard of care treatment modalities (chemotherapeutic/targeted agents and radiation), from which the dose that is effective to reduce tumor burden with minimal side effects is known, with a small molecule inhibitor against γ-secretase thus preventing NOTCH protein activation. Despite the great availability of different efficient NOTCH inhibitors, there are none that are FDA-approved. However, several inhibitors have been tested in human clinical trials and side effects are known. This prior information would greatly facilitate further designing and development of clinical trials, reducing costs, and speedy implementation in the clinic in case of superior outcome to that of standard of care. There is a plethora of treatment options that are efficacious for the target they were designed for, however, due to a suboptimal clinical trial design, the complete efficacy of the drug upon the tumor burden has not been accurately evaluated. A clear example of this are the NOTCH inhibitors, which have not been successful in reducing significantly tumor burden when delivered as a monotherapy on an unstratified patient population. Our study gives evidence that when NOTCH inhibition treatment is delivered in NOTCH-expressing NSCLC or GBM multicellular spheroids *in vitro*, and in ectopic xenografts *in vivo*, both NSCLC and GBM growth is delayed, an effect that is further potentiated in combination with chemoradiation. Further studies are needed to substantiate our findings, but the results so far are encouraging. Moreover, it is imperative to identify companion biomarkers to assess, prospectively, the efficacy and or toxicity of the drug over the course of the treatment, enabling therapeutic regimen redesigning if need be. Here we suggest the use of TLSP as a potential companion biomarker, and although its validation still needs some more time and resources, money in the discovery of companion biomarkers would be well spent since they can aid tremendously in therapeutic assessment, and potentially reduce the amount of trials needed.

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Innovation

The concept of combining chemotherapy or radiation with NOTCH inhibitors is not new, there are several ongoing clinical studies in different cancer types,

hematological and solid tumors including lung, breast, pancreas, colorectal, and brain cancers. However, what is novel is the triple combination of chemoradiation with NOTCH inhibitors, which is what we propose in both NSCLC and GBM models.

In order to obtain optimal clinical translation, the most physiologically relevant models and clinically sound treatment options must be incorporated in preclinical studies. Our lab has invested a great amount of resources in obtaining more reliable models. The use of 3D cancer spheroid models to test the efficiency of treatment combinations in long-term *in vitro* is slowly starting to supersede the more common 2D assays which monitor efficacy in short-term assays. *In vivo*, the use of orthotopic models and the application of treatment planning software (SmART-Plan) to precisely deliver image-guided radiotherapy using cone beams that can go as low as 1-mm in precision, are novel models and technologies that more closely mimic the clinical setting compared to the ectopic xenografts models which are more widely used.

Market Opportunities

Current standard of care treatments for both advanced stage NSCLC and first line treatment for GBM, both consisting in a combination of chemotherapeutics/targeted agents and radiation, are giving rise to tumor recurrence. Therefore, there is a need to carefully and thoughtfully design treatment strategies for such patients to improve prognosis and prolong survival. In this thesis we have shown evidence that NOTCH-based treatments are promising in such a respect even though there are still further preclinical studies to be addressed before considering progression into clinical trials such as addressing the effect of the triple combination (chemoradiation and NOTCH inhibition) in orthotopic models for NSCLC, and assessing the effect of the triple combination in immune proficient mice for both models. The observation that the triple therapy (chemoradiation and NOTCH inhibition) was more effective than chemoradiation for several chemotherapeutic agents and targeted agents in NSCLC and in GBM, is suggestive that other cancers with active NOTCH signaling may benefit of such treatments as well.

NOTCH activating mutations occur in ~50% of T-ALL tumors, however in solid tumors the mutation rate is much lower. In this thesis we have shown that although tumors with NOTCH overexpression are more responsive to NOTCH inhibition therapy, NSCLC tumors with wildtype levels of NOTCH signaling activation, also show significant tumor growth delay with respect to placebo. This suggests that a greater patient population could benefit from the proposed regimen approach.

In conclusion, if patients that are susceptible to NOTCH inhibition therapy are recognized, companion biomarkers are identified, and the effect of the immune system potentiates, or at least is not detrimental, for the treatment strategy here proposed, it is highly likely that hematological and solid tumors receiving chemotherapeutic, targeted agent and/or radiation treatment will benefit from the addition of NOTCH-based therapies to prolong survival in cancer patients.