Drug missiles for cancer treatment

Dr Lali K Medina-Kauwe is researching the mechanisms of pathogen proteins; here, she discusses how her findings may help improve tumour targeting techniques, and is optimistic about the expected applications for patients.

Firstly, could you outline the primary objectives of your research and its intended outcomes?

The primary objectives of my research are the investigation of cell penetration and payload delivery mechanisms of pathogen proteins, and their exploitation for the overcoming of certain cellular barriers to efficient gene and drug transfer. My immediate intended outcome from these studies would be to develop technologies for cancer therapy.

Could you explain why doxorubicin has been so important in the treatment of cancers since its inception in the 1960s? What problems are there with this drug and how does your research seek to overcome them?

Doxorubicin is one of the most established drugs in clinical use for cancer treatment. However, it can adversely kill healthy tissue and cause heart damage. Our research seeks to improve therapeutic efficacy and safety through a unique and potentially improved method compared to most other drug-targeting strategies. My lab’s technologies are not only designed to target tumours but also to overcome cellular barriers by utilising the minimal components of viruses to penetrate target cells for the effective delivery of therapeutic payloads.

What is the role of viral capsid proteins in your work and what properties do they possess which make them appropriate ‘vessels’ for doxorubicin to reach cancerous cells? What is the penton base and its role within the virus?

The penton base is one of the three types of protein that make up the capsid or shell of the adenovirus. It facilitates the binding of the virus to cell surface attachment proteins leading to the cell’s internalisation of the virus. The penton base acts as a mediator by penetrating through the vesicle membrane in which the virus is encased, thus allowing the virus to access the cell nucleus where viral genes can be transcribed for the production of new viruses. Through genetic engineering, I have produced a synthetic penton base that recapitulates the cell binding, cell entry, membrane penetration, and nuclear delivery activities of the whole virus without other viral constituents. This penton base is capable of delivering a payload through similar routes without using whole viruses for therapy.

Could you explain how you came to synthesise the HerDox nanoparticle? What is this nanoparticle comprised of and what qualities enable it to act as a ‘missile’ for targeted drug delivery?

HerDox was developed rather unexpectedly. The engineering of the penton base protein was originally intended to deliver nucleic acids for the non-viral gene therapy of breast cancer. I was comparing cancer cell death by gene therapy agents to Doxorubicin when it occurred to me that the latter could be delivered unaltered by my gene delivery vehicles through its well-known DNA-binding activity.

HerDox consists of three components: doxorubicin, a small piece of double-stranded nucleic acid, and the HER-targeted penton base known as HerPBK10. These three constituents can enable self-assembly into small, tumour-targeted particles without the need of chemical modification.

How much clinical potential does this technique have in changing cancer treatment? Furthermore, how much commercial potential might it have – and how far away might clinical trials and a practicable treatment be?

We have already carried out initial tests on storage stability which showed the drug’s lasting potential. In addition, HerDox can be easily manufactured and does not require highly sophisticated instrumentation. Moreover, as it does not have any detectable effect on the heart, HerDox could be used as an improved tumour-targeted treatment. Clinical trials of the drug will follow additional preclinical studies in drug-resistant models.

Is there potential for this style of treatment – using viral proteins to deliver drugs to targeted tumour cells – to be applied to other cancers, or in other diseases?

As described earlier, HerDox has been tested on HER2+ breast cancer models in vivo and has proved efficient on HER2+ glioma tumour cells in vitro. We will extend testing to HER2+ androgen-independent prostate and ovarian cancer. We are also planning to re-engineer HerPBK10 by replacing the HER-targeting segment with a short protein region targeted to cell surface proteins found on lung cancer cells. As recent studies have shown, the environment surrounding the tumour facilitates its progression. So, we are going to explore different methods of targeting HerDox-like complexes to cells that make up the tumour micro-environment. We will eventually investigate the possibility of targeting cancer stem cells responsible for tumour recurrence.
**Improved efficacy, fewer side-effects**

Research at Cedars-Sinai Medical Center is exploring an innovative nanotherapeutic approach to specifically target cancerous cells in a molecular missile fashion, paving the way to safer cancer treatments.

**SINCE THE LATE 1960s,** the anthracycline ‘doxorubicin’ – a toxic agent to dividing cells – was used as the main therapeutic drug in cancer treatment. The problem however, lay in its delivery to localised and remote tumours, which, since the inception of the drug, was affecting both diseased and healthy cells. Primarily, it was the drug’s heart-damaging side effects that raised considerable concern, directing research towards synthesis of new forms of the drug. These new forms, used either as an antibody-targeted drug or enclosed in liposomes, can reduce significantly the high doses needed for tumour cell death, thereby lessening its cardiotoxicity. Nevertheless, this approach is limited in physiological stability, cellular uptake, and release into tumours. Improving cancer treatment by specifically targeting tumour cells without affecting healthy tissue is at the core of Dr Lali K Medina-Kauwe’s lab work – developing new methods for the delivery of doxorubicin to tumours.

Medina-Kauwe utilises the cell binding, membrane penetration and intracellular trafficking functions of pathogen proteins to develop novel cell-targeted nanotherapeutics. The chosen approach is to study the cell entry processes of adenovirus (Ad) capsid proteins as a guideline for the design and engineering of nano-viral nanoparticles that can mimic the high efficiency cell entry mechanism of the virus, thus avoiding the safety risks associated with using whole viruses for therapy. The lab has enhanced these molecules even further by converting them into molecular missiles to target specific cancer cells for destruction without affecting non-target cells. It is currently testing these molecules for targeting therapy to HER2+ breast cancer in an animal model of breast cancer. These molecular missiles are being assembled for delivering different types of therapeutic payloads to cancer cells such as genes, drugs, or small interfering RNA (siRNA) with which mutant or cancerous cells can be suppressed.

More importantly, the Medina-Kauwe lab is studying vector-cell interactions like intracellular trafficking of viral capsid proteins for the identification of molecular and cellular barriers to efficient cell penetration and accumulation at intracellular targets.

Thus far, the team has discovered multiple alternative cell entry pathways that may be used by the same capsid protein, as well as novel routes that could be exploited for therapeutic cell entry. The above studies describe the particular roles that certain capsid proteins play in virus pathology, and also facilitate the design of safer delivery agents derived from minimal components of the viral capsid.

**THE SYNTHESIS OF PENTON BASE**

Medina-Kauwe has studied the self-assembly, cell binding, and cell penetration activities of the proteins that make up the outer shell of many viruses, and has engineered one such protein, the penton-base, for the delivery of therapeutic agents to tumour cells without using the rest of the virus. The penton base lies at each vertex of the 12-sided outer shell of the adenovirus, and functions as a mediator between several important early steps in the viral infection mechanism. It also facilitates penetration of the virus through the cell membrane, a major barrier to the delivery of a payload to the cell interior.

The significance of cell membrane penetration first became apparent to Medina-Kauwe when she was synthesising artificial penton base proteins for the delivery of nucleic acids to tumour cells as a non-viral gene therapy approach to cancer treatment. DNA cannot breach the cell membrane without the help of carrier molecules that can disrupt the double-layer of lipids of this cellular barrier. Studying viral early infection mechanisms can present ways to overcome such barriers for improved payload delivery, as viruses have developed means to transport their DNA across several cellular obstacles to reach the cell nucleus for viral gene expression and propagation.

Medina-Kauwe’s synthetic penton base can assemble itself with nucleic acid and recapitulate the early infection steps of the whole adenovirus. She has modified these proteins to specifically penetrate tumour cells, taking advantage of the DNA-binding features of doxorubicin to capture this drug in nano-sized particles, consisting of modified penton base protein assembled with nucleic acid. The nanoparticle that results from this process, named HerDox, has got the features of a tumour-targeted molecular missile for chemotherapy delivery.

**RAISING THERAPEUTIC POTENTIAL**

The human epidermal growth factor receptor, or HER, consists of two subunits (HER2/HER3 or HER2/HER4) that transmit cell maintenance and proliferation signals from the cell surface to its interior. HER2+ tumours are characterised by the amplification of the HER2 subunit which gives an aggressive cell-growth characteristic prone to metastasis. While nearly 25 to 30 per cent of breast cancers are classified as HER2+, the elevation of HER2 can also cause the progression of androgen-independent prostate cancer, ovarian cancer and glioblastoma. The cells’ mutations may prevent them from responding to certain therapies intended to block HER2 signalling. This might explain the resistance that has been observed in up to 70 per cent of HER2+ breast cancer cases treated with signal-inhibitors such as Herceptin®.

HER2 signalling is also important for the maintenance of heart muscle which shows normal levels of HER2. However, HER2 inhibitors cannot make this distinction. As a result, HER2 signal-blocking in the hearts of breast cancer patients treated with Herceptin® has led to cardiac damage exacerbated by doxorubicin.

HerDox is designed to target HER2+ tumours, which display elevated levels of HER2, and avoid tissue displaying normal HER2 levels. The Medina-Kauwe lab has found that HerDox delivery in mice targets and kills HER2+ tumours at a dose over 10-times lower, compared to doxorubicin while sparing the heart. HerDox is designed to capture the drug, unmodified, into virus-like particles whose cell targeting and penetration activities remain uncompromised by chemical modification. This nanotherapeutic approach increases the drug’s efficacy and safety as it preserves the latter’s potency while directing its delivery and release into target tumours. This is the reason why a lower dosage can be used while still retaining its tumour-toxicity.

**COLLABORATION AND FUTURE ASPIRATIONS**

There are currently three full-time scientists and two volunteers working in the Medina-Kauwe lab. In addition, four other scientists have contributed to the work with their expertise in specialised procedures such as imaging and echocardiography in small animals. Two of them are Dr Daniel Farkas and Dr Behrouz Sharifi at Cedars-Sinai Medical Center. Because of the multidisciplinary nature of the development and testing of HerDox,
FIGURE 1. Summary of HerDox assembly and mechanism. HerDox self-assembly is based on known interactions of both doxorubicin (Dox) and carrier protein (HerPBK10) with DNA, and results in the formation of ~10 nanometer diameter round particles (viewable by cryo-electron microscopy, or cryoEM) that are stable in blood and exhibit tumour-preferential targeting in mice that is governed by receptor level. Receptor-mediated internalisation facilitates cell entry while the penton base segment of HerPBK10 mediates penetration into the cell interior where Dox is released from HerDox.