Venom's verdict for brain tumours

B. Sandhya Rani

The quality of life and overall life expectancy of patients diagnosed with malignant brain tumours are dismal in spite of significant advances made in the development of new cancer therapies. Surgery is the first line of treatment adopted in the brain tumour terrain, followed by chemotherapy and radiation. The brighter side of this avenue is, if the tumour is resected entirely, the chances of relapse of the tumour are reduced, as resection correlates with recurrence, metastasis and overall survival rate[1]. A surgeon entering the operation theatre is always hopeful of a complete resection, but the scalpel in his hand is the 'Achilles heel' which has the power to cure, but if placed on a normal tissue, can inflict irreparable damage to the eloquent brain.

Neuro-imaging is currently the gold standard modality used by neurosurgeons for pre-operative navigational planning; however, its usefulness is limited by the infiltrative nature of the tumour and brain shift (midline shift) which occurs during the surgical procedure. Hence there is a critical need for real-time image-guided intra-operative approaches that would allow tumour boundaries to be effectively visualized and accurately demarcated live during neurosurgery. Such tools have shown great promise in the clinics, to help guide surgeons in removing as much malignant tissues as safely as possible, while sparing the surrounding normal cortex.

In this scenario, the good news for surgeon's scalpel comes from the work by James Olson and his colleagues at Fred Hutchinson Cancer Research Center, Seattle Children's Hospital, and the University of Washington, who have developed the technology platform called Tumor Paint[2]. Pre-clinical studies indicate that Tumor Paint, injected intravenously as a single dose before surgery, lights up specifically the cancer cells and hence circumvents the limitation of blurred demarcation between the normal brain cortex and the tumour tissue.

Tumor Paint is a bio-conjugate of synthetic cholorotoxin (CTX) and fluorescent molecule Cy5.5. CTX is a small peptide initially purified from the paralysing venom of the 'Israeli deathstalker' scorpion[3] in 1993. CTX exhibits several advantages as a ligand for diagnosis and treatment of brain tumours. It shows enhanced tropism to tumours and minimal likeability to normal tissue. It has no known immunogenicity and clinical trials to date, conducted with CTX, indicate that it has negligible toxicity in humans and shows no antibody response. For reasons that remain unclear, it can cross the blood-brain barrier (BBB) effectively. Lastly, CTX can be re-engineered from *Escherichia coli* by recombinant DNA and protein purification techniques. These unique features of CTX have been exploited by the scientific community to develop bio-conjugates used in intra-operative imaging technologies. Though the molecular mechanism of the interaction of CTX specifically with cancer cells is still elusive, a few studies have reported that CTX latches on to cancer cells by interacting with matrix metalloproteinase-2 (MMP-2), receptor-associated chloride channel, glioma-specific chloride channel (GCC)[4] and annexin-A2 (ref. 5), which are specifically expressed in the membranes of glioma cells but not in normal human cell membranes.

CY5.5 is a fluorescent molecular radar that emits photons in the near-infrared (NIR) spectrum. NIR dyes are excited and emit photons in the spectrum of 650–900 nm, allowing deeper light penetration through tissues as well as decreased light scattering and autofluorescence. This schema enables good signal-to-noise ratio and sharp definition of tumour boundaries[6]. Bringing both molecules together resulted in the compound CTX : CY5.5. The molecular beacon was injected intravenously in mice with glioma xenografts. The eureka moment came when in less than an hour, the tumour glowed, brightly and distinctly from the rest of the mouse (Figure 1 *a*). Olson and his colleagues had potentially hit upon the magical bullet that lights up cancer and brings in smile to surgeons and hope to patients.

The bio-conjugate that would specifically tag cancer cells and make them glow fluorescent under laser light was illustriously named 'Tumor Paint' which caters to maximal tumour removal. As seen in Figure 1 *b–d*, fluorescence from BLZ-100, the current clinical candidate, was able to show otherwise undetected metastasis, which is further substantiated in H&E staining. Hence the use of BLZ-100 for detecting micrometastases during surgeries holds appeal.

Preclinical studies indicate that CTX-based Tumor Paint can also be used in other malignancies, such as breast, neck, colon melanoma and lung cancers to increase the likelihood of detecting micro-metastasis, thus indicating the far-reaching clinical ramifications of Tumor Paint technology.

Tumor Paint has been tested in rodents and canines as a part of the preclinical studies[2,7]. These studies reported that 'Tumor Paint' exhibits increased specificity, sensitivity and no worrisome toxicity and longer duration of probe activity, which permits imaging long after peak serum levels have subsided.

In light of the promising results obtained from studies with CTX : CY5.5, Olson wanted to take his invention to the clinic. This desire led him to co-found the biotechnology enterprise 'Blaze Bioscience', which is the pioneering work of taking Tumor Paint technology to the clinics. BLZ-100 is the first lead candidate incorporating Tumor Paint technology, that combines the targeting peptide CTX and a fluorescent beacon indocyanine green (ICG). The safety and efficacy of ICG have been tested previously in clinics in the arena of vascular imaging and surgeries[8].

BLZ-100 has been reported to be potent enough to detect cancer cells which count to a few hundred in number under standard imaging systems that use NIR spectrum. To refine the technology further and enable visualization of low levels of the dye attached to the tumours, researchers at Cedars-Sinai Medical Center in Los Angeles have developed an experimental tumour-imaging system. This uses a standard charge-coupled device (CCD) 2-in-1 camera system, which simultaneously captures white light and NIR images, and combines these images on a high-definition video monitor. This system is optimized to the concentration and amount of ICG in this product and is capable of visualizing concentrations of ICG down to 1 nM and BLZ-100 to well below 1 nM (extinction

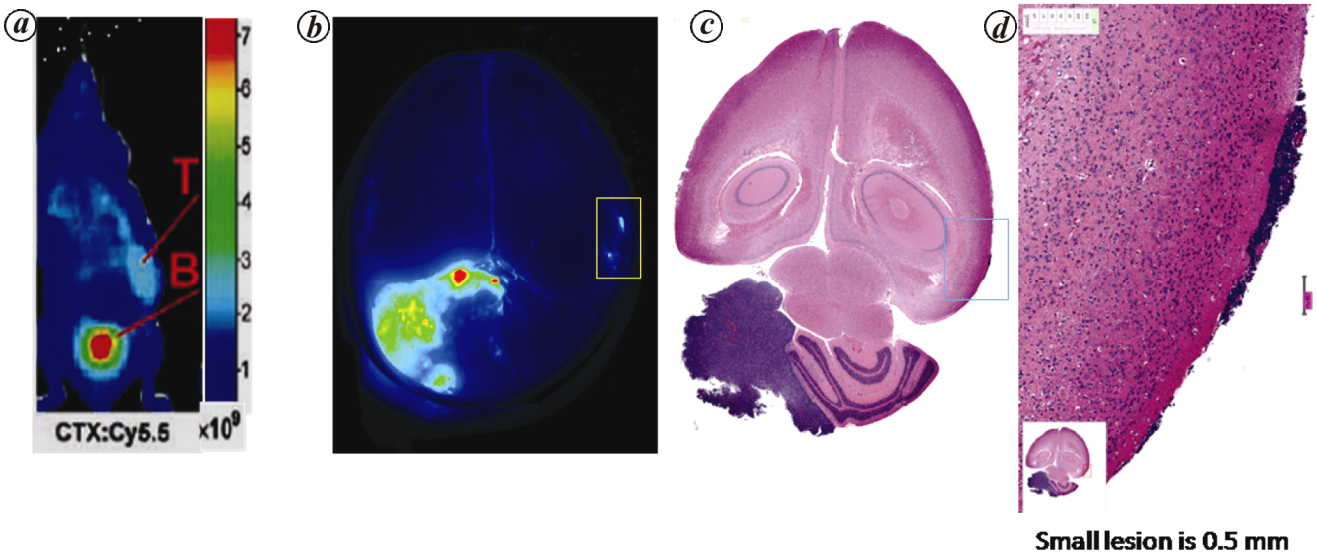

Figure 1. ***a***, Biophotonic imaging of glioma in mouse in CTX : CY5.5 binding to tumour (T) and renal excretion seen in the bladder (B). ***b***, *In vivo* near-infrared fluorescence image of intravenously administerd BLZ-100 to mice with spontaneous medulloblastom. ***c***, ***d***, Similar image analysed by H&E staining. (Courtesy: Blaze Bioscience Inc, Seattle, WA, USA.)

at 100 pM), thus increasing the sensitivity and specificity of BLZ-100 fluorophore[9].

With Tumor Paint technology still in the early stage clinical trials, the day might not be far when a surgeon can see and remove tumours completely, giving patients extended life span or the possibility of complete cure. Truly the invention of Tumor Paint is a saga which underscores the importance of curiosity, inquisitiveness, conviction and will to do translational science.

Kudos to the scientists who thought that 'venom can be a life-saving ambrosia'.

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B. Sandhya Rani, S. Ramaseshan Fellow.
e-mail: bsandhya163@gmail.com