Venomtech

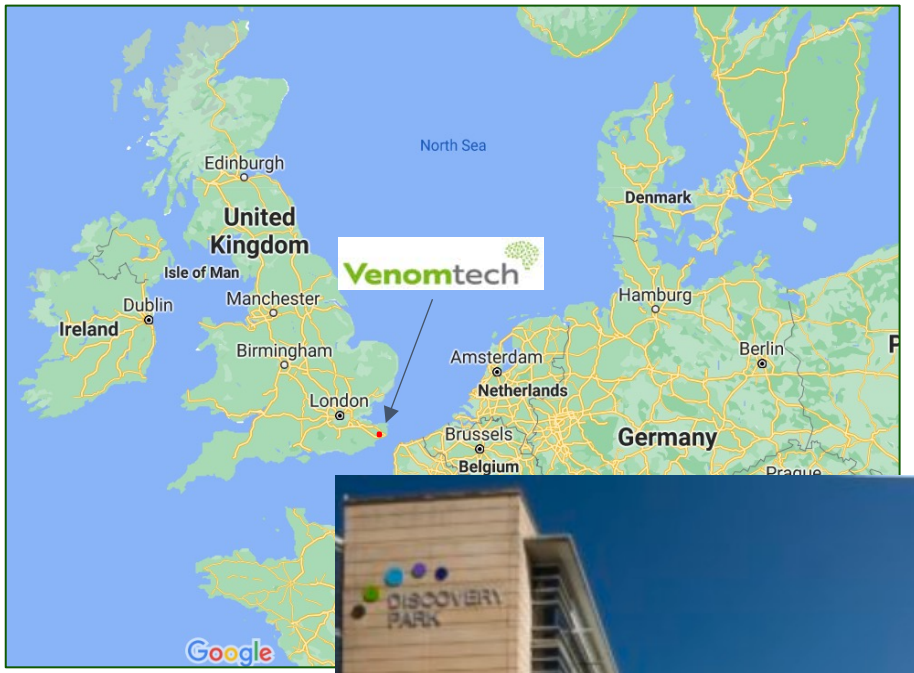
Examining the synergistic effect of venoms to enhance chemotherapeutics and irradiation using high content cell imaging

Steve Trim founder and CSO
Venotmech Ltd – s.trim@venomtech.co.uk

INTRODUCTION TO VENOMTECH AND VENOM COMPOUNDS IN DRUG DISCOVERY

About **us**

- ☑ **A global leader in venom research, based in Kent, UK.**
- ☑ **Unlocking the potential of millions of years of evolution**
- ☑ **Whole venoms from verified Nagoya exempt sources**
- ☑ **Custom-designed, targeted, fractionated venom arrays**
- ☑ **A variety of molecular targets, with a high degree of selectivity:**
  - **Ion channels**
  - **GPCRs**
  - **Enzymes**
- ☑ **Successful even on hard-to-hit targets or where traditional approaches failed**
- ☑ **Resulting in purified peptides for leads and/or controls**



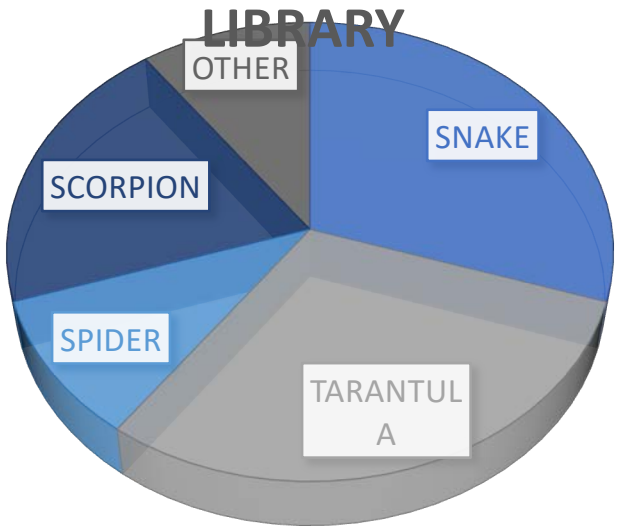
The Venomtech **library**

Library size

- 178 species – all Nagoya protocol exempt
- Approx. 500 animals
- Up to 200 fractions per venom

Library composition

- 30% snake
- 30% tarantula
- 10% spider
- 20% scorpion
- 10% other (jellyfish, insects, etc.)



Venom composition

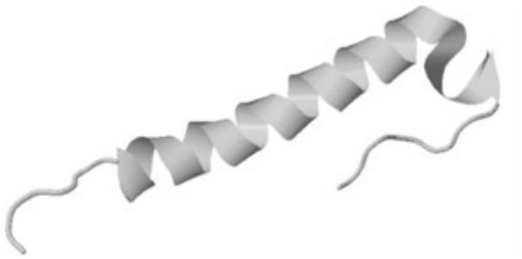
- Small molecules such as acylpolyamines
- Peptides – mostly 1-10 kDa, but some larger
- Large proteins

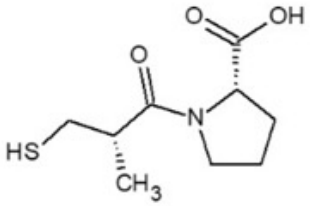
Peptides

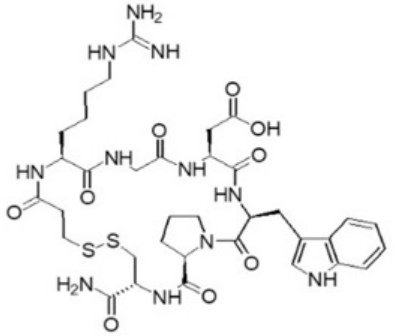
- Some linear
- Some with stable folds
- Many cell penetrating

20,000 peptides, proteins and small molecules available – the UK's largest library of venom-derived compounds.

Venom-**based medicines**







GLP-1 agonist – derived from lizard venom (Gila monster)

1st Anti-hypertension drug – mimics the action of Bradykinin potentiating peptides found in snake venom

Anti-coagulation drug that acts on platelets (derived from rattlesnake venom)

Chronic pain relief drug – contains Ziconotide - derived from cone snail venom

Venoms in chemotherapeutic work

Case study : 2D **cancer cell cytotoxicity**

T-VDA^{ctx}

Diversity: 1
Fractions: 303
TQ: 4
Hit Rate: 2%
Z' - 0.85

Step 1 - screened against two cancer cell lines (BxPC-3 pancreatic cancer and SW620 colorectal cancer cell lines)

Plate 1

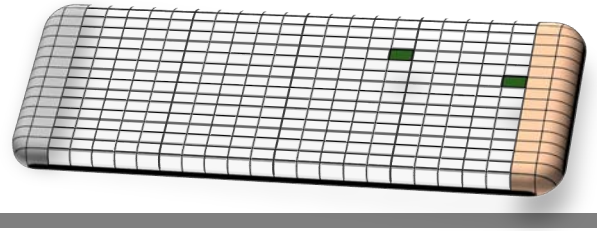
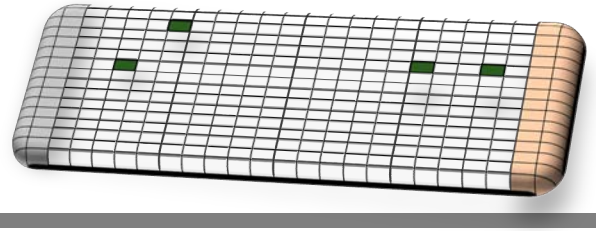
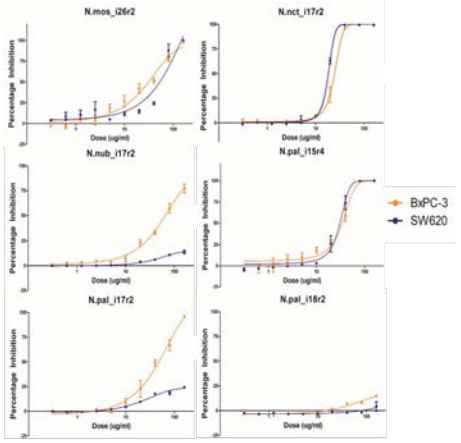


Plate 2



Step 2- Hit confirmation



Step 3- Hit ID (MS/MS)

Sample name	Uniprot ID	Protein name	Sequence coverage
N.nub_i17r2	P01456	Cytotoxin 1	43%
N.nct_i18r2	P01452	Cytotoxin 4	40%
N.pal_i15r4	P01468	Cytotoxin 1	65%
N.pal_i17r2	P01456	Cytotoxin 1	43%

N.nub_i17r2 sequence identification

Protein sequence coverage: 43%

Matched peptides shown in ***bold red***.

1 LKCHKLVPFV KKTCPEGKNL CYKMFMVSTS TVPVKRGCID VCPKDSALVK
51 YVCCSTDKCN

N.pal_i17r2 sequence identification

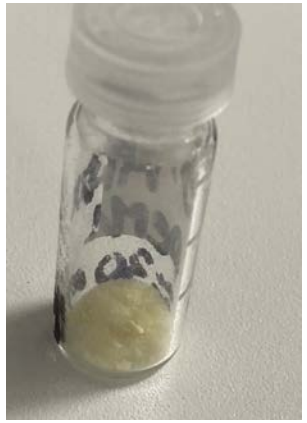
Protein sequence coverage: 43%

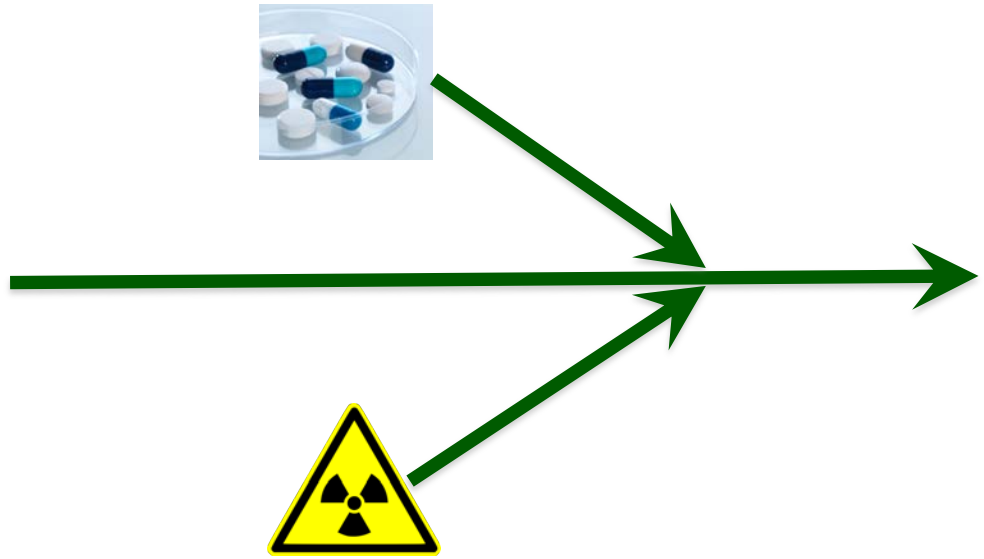
Matched peptides shown in ***bold red***.

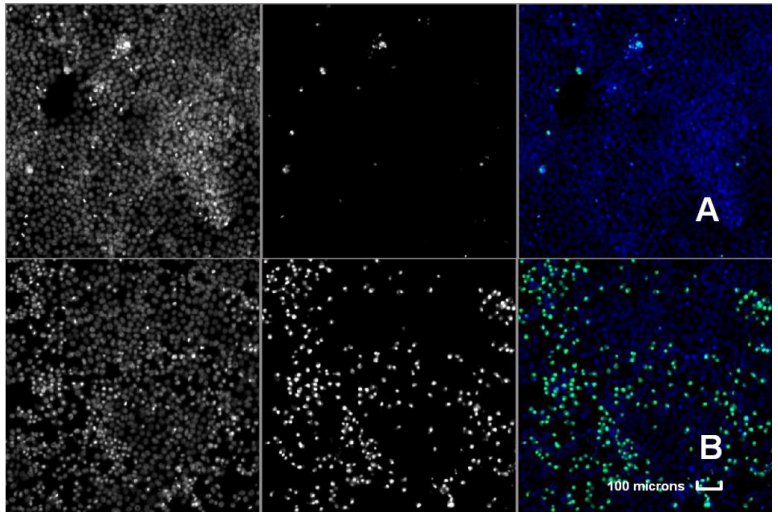
1 LKCHKLVPFV KKTCPEGKNL CYKMFMVSTS TVPVKRGCID VCPKDSALVK
51 YVCCSTDKCN

Screening the T-VDA^{ctx} has identified cobra venom peptides with previously undiscovered selective preference for inhibiting pancreatic cancer cells. The sequences of these peptides are also potentially novel and thus would not have been easy to discover without screening of the T-VDA^{ctx}. Interestingly discovery not all the hits are selective even though the peptides are closely related.

Summary of **experimental method with Imagen**







1

Diversity of venom collection

• Phylogenetic and geographical diverse
• 3 cobra venoms
• 4 viper venoms
• 1 theraphosid spider venom

2

Cell culture and dosing

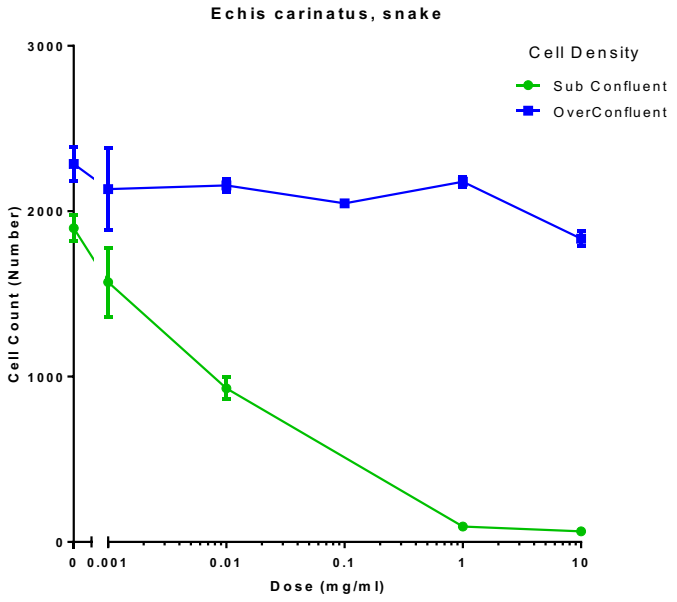
• SKOV3 Ovarian cancer cells in 2D
• Patient derived glioblastoma in 3D
• +/- whole venom
• +/- Etoposide or Paclitaxel
• +/- radiation therapy

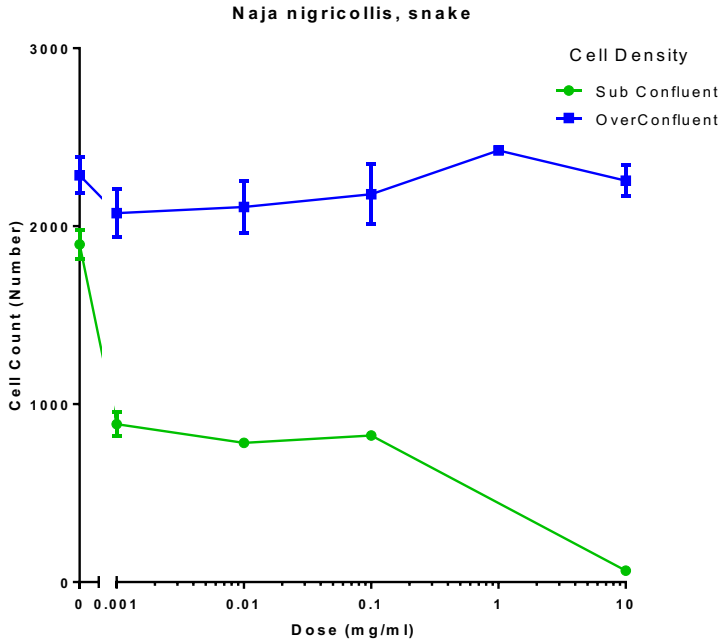
3

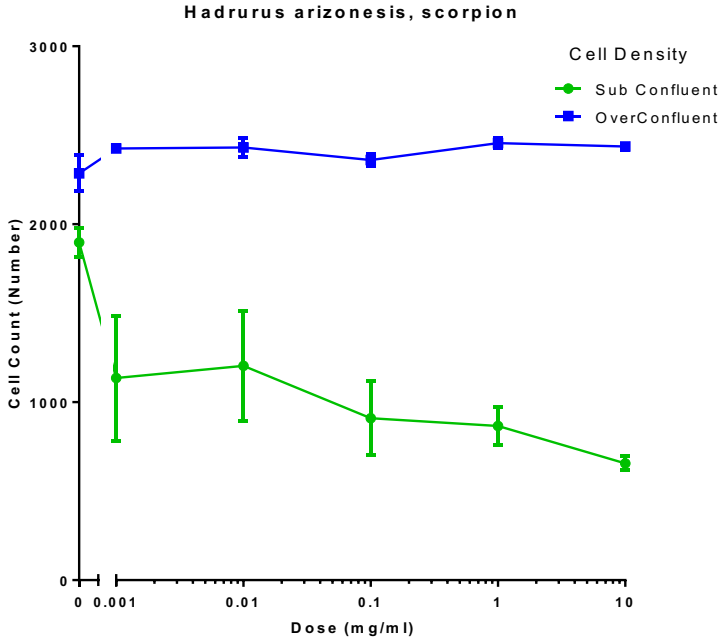
Data collection and analysis

• Draq7 and Hoechst staining
• ArrayScan VTI HCS Reader (Cellomics)
• Cell number and spheroid size
• Cytotoxicity
• Caspase 3 activity

Results – **SKOV3 in 2D**





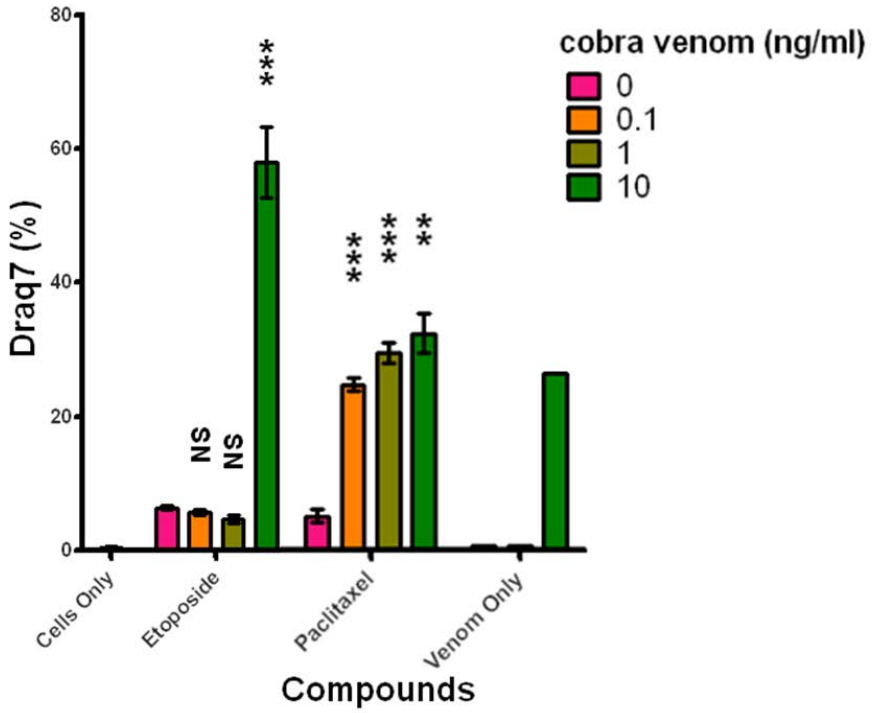


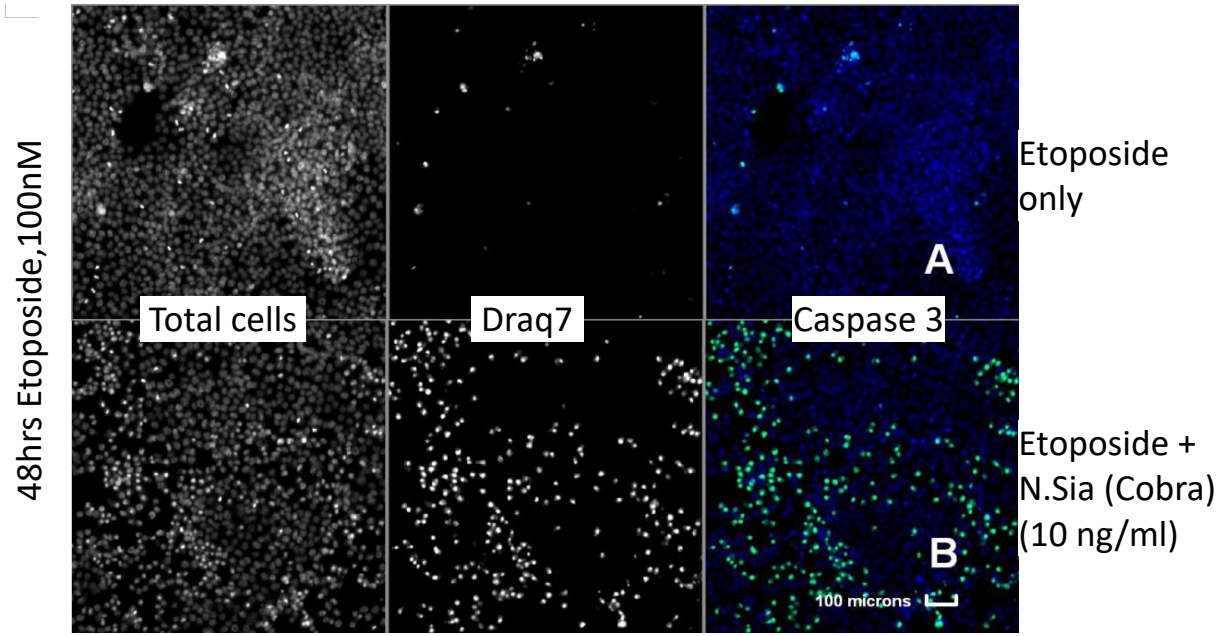
1

Venom effect on SKOV3

- cytotoxicity is dose dependent
- confluent cells are resistant
- Scorpion venom cytotoxicity does not increase Draq7 staining (not shown)

Results – **SKOV3 in 2D**





2

Venom effect on SKOV3

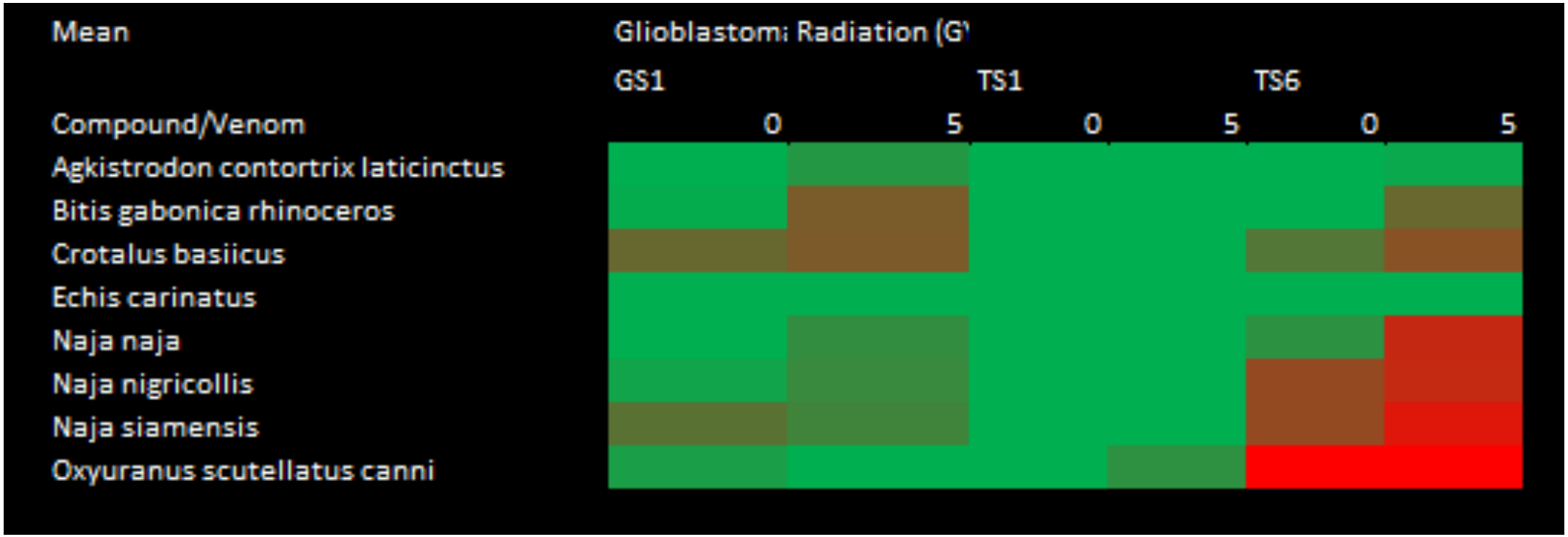
- Naja siamensis venom is non-toxic below 10ng/ml
- Chemotherapeutics have low cytotoxicity at 100nM
- Cytotoxic synergy is venom dose dependent

3

Synergistic cytotoxicity images

- combined therapy damages membrane integrity
- Cell death appears to be through apoptosis due to Caspase 3 activation

Results- **Glioblastoma 3D**



4

Venom effect on patient derived glioblastoma spheroids

- Patient selectivity seen in snake venom dosing at low doses 0.01ng/ml
- Vipers are less toxic than Elapids
- several venoms increased sensitivity to irradiation in 2/3 patient lines

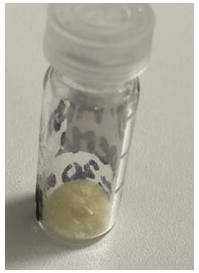
Below 3 Percentile	
Above 49 Percentile	

Summary of **Venom cancer data**

1

Cobra cytotoxins

• Venoms are complex mixtures which can be separated
• Cobra venoms contain cytotoxins that can be selective
• Further work is required to see if these toxins are responsible for the other cancer cell cytotoxicity and chemotherapy synergism



2

Cell density dependent cytotoxicity

• First identification of venom cytotoxicity dependance on cell density
• Sub-confluent cells are dividing faster
• Specificity to rapidly dividing cells is a key mechanism in cancer treatment

3

Venom- chemotherapy synergy

• Dual dosing with venom and chemotherapy increases observed cytotoxicity
• Paclitaxel and Naja siamensis venom had the greatest effect on Draq7 at the lowest doses
• Synergistic cytotoxicity could reduce doses needed
• Further work is required to see if this synergy is restricted to cancer cells

4

Venom synergy with irradiation

• Patient derived glioblastomas have differing sensitivity to low dose venoms
• As expected (by Venomtech) elapid venoms are more toxic
• Venom doses of 10pg/ml enhances the cytotoxicity of irradiation
• We need to understand the mechanism and the venom components involved to understand if this could reduce the patient radiation dose needed

Venomtech

THANK YOU FOR LISTENING

Contact me –
Steve Trim CSO – s.trim@venomtech.co.uk
Office landline – 01304 892694

13 YEARS ANNIVERSARY

Special thanks to Gareth Griffiths (and others) from Imagen Biotech for the cell work and getting the data together for our SLAS poster ’22

Thanks to Dr Carol Trim (CCCU) for allowing me to used the Pancreatic cancer cell data generated by Dr Emily Knight