Targeting Breast Cancer Signaling Pathways with Animal Venoms

Danielle McCullough*, Cristina Atofanei*, Emily Knight*, Steve Trim$, Carol Trim*

*School of Human and Life Sciences, Canterbury Christ Church University, Canterbury, Kent, CT1 1QU

$Venomtech Ltd. Discovery Park, Sandwich, Kent, CT13 9ND

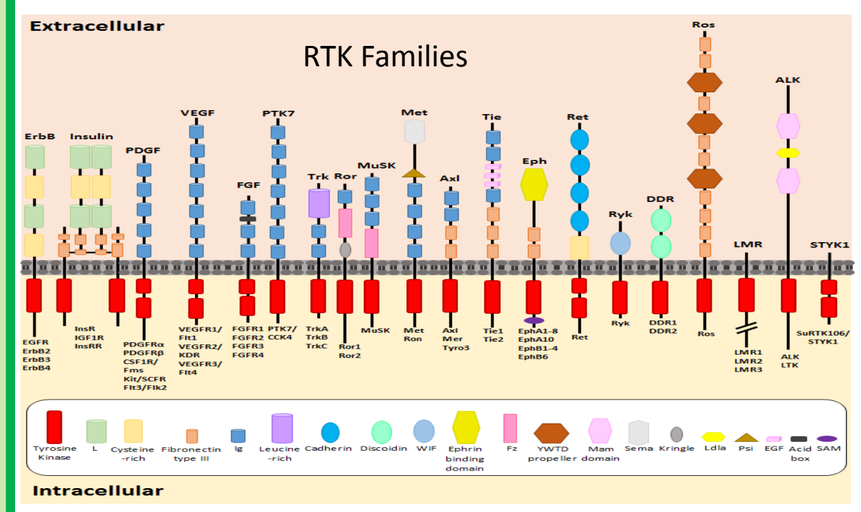
Receptor Tyrosine Kinases: (The EGF Family)

•There are more than 50 receptor tyrosine kinases (RTKs), which belong to at least 18 receptor families and 32 non-receptor tyrosine kinases (nRTKs)

•All RTKs contain large glycosylated extracellular ligand-binding domains, a transmembrane region and a cytoplasmic domain (composed of a catalytic tyrosine-kinase, a juxtamembrane region and a carboxyl-terminal tail)

•**EGFR** *(ErbB-1),* **HER2** *(ErbB-2),* **HER3** *(ErbB-3),* and **HER4** *(ErbB-4)* belong to the Epidermal Growth Factor family of receptors (ErbB). The ErbB RTK family are activated by a subset of 11 potential growth factor ligands. HER2 has no ligand binding domain whilst HER3's tyrosine kinase has no catalytic activity.

•EGFR family members are over-expressed in many aggressive cancers, including breast, gastric, endometrial, colorectal, pancreatic and non-small cell lung cancer (NSCLC)



Methods

Crude Venom Western Blots:-

- Crude venoms were diluted in DMEM(FCS (10%), L-Glutamine (1%), Pen-Strep (1%)) to produce 1/50, 1/100, 1/1000, 1/10,000, 1/100,000, 1/1,000,000 final dilutions **(A-F respectively)**
- Cells grown to 80% confluency, treated with venom dilutions for 2hours, then stimulated with EGF for 5minutes
- Cell lysis, Gel Electrophoresis, Western Blot detection (of EGF, Actin and Tubulin) and Enhanced Chemiluminescence using Biorad Chemidoc were undertaken **(Fig. 1)**

Fractionated Venom Western Blots:-

- Lysophiled venom fractions reconstituted in nuclease-free water. All fractions diluted in DMEM(FCS (10%), L-Glutamine (1%), Pen-Strep (1%)) to produce 1/50 final dilution. Remaining method undertaken as above **(Fig. 2)**

Kinome Array Analysis:-

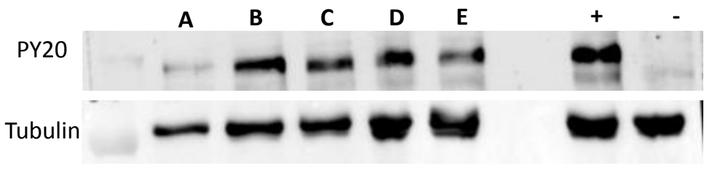
- Crude venom western blots undertaken to determine venom dilutions used for Kinome Array Assay. Final selected dilutions in table.
- Kinome Arrays undertaken as via provided protocol (R&D Systems), Imaged using Biorad Chemidoc and analysed using Biorad Image Lab software **(Fig. 3 and 4)**

Venom	Protein Conc. (mg/ml)	Dilution
Theraphosid 1	258.80	1:100
Scorpion	101.95	1:50
Buthid	216.00	1:150
Elapid	280.98	1:10,000
Elapid	261.42	1:10,000
Crotalid	66.37	1:1000

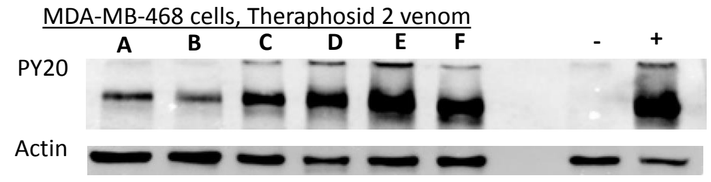
Results and Data

Figure 1:- Crude Venom Western Blots:-

MDA-MB-468 cells, Theraphosid 1 venom



MDA-MB-468 cells, Theraphosid 2 venom



A431 cells, Scolopendra venom

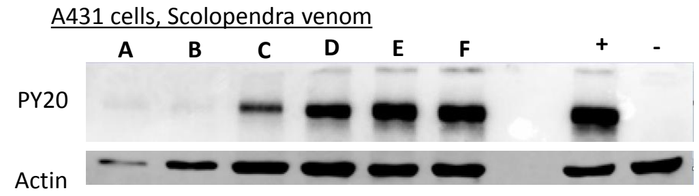


Figure 2:- Fractionated Venom Western Blots:-

MDA-MB-468 cells, Theraphosid venom 2

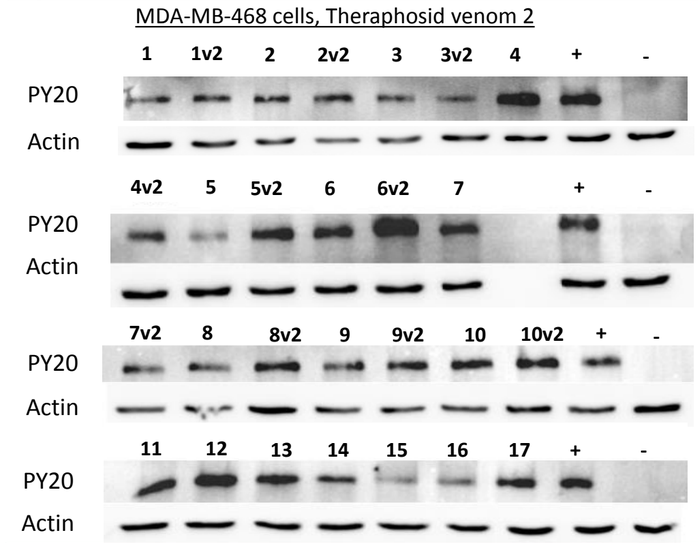


Figure 3A: Kinome Arrays:-

MDA-MB-468 (45s Exposure)

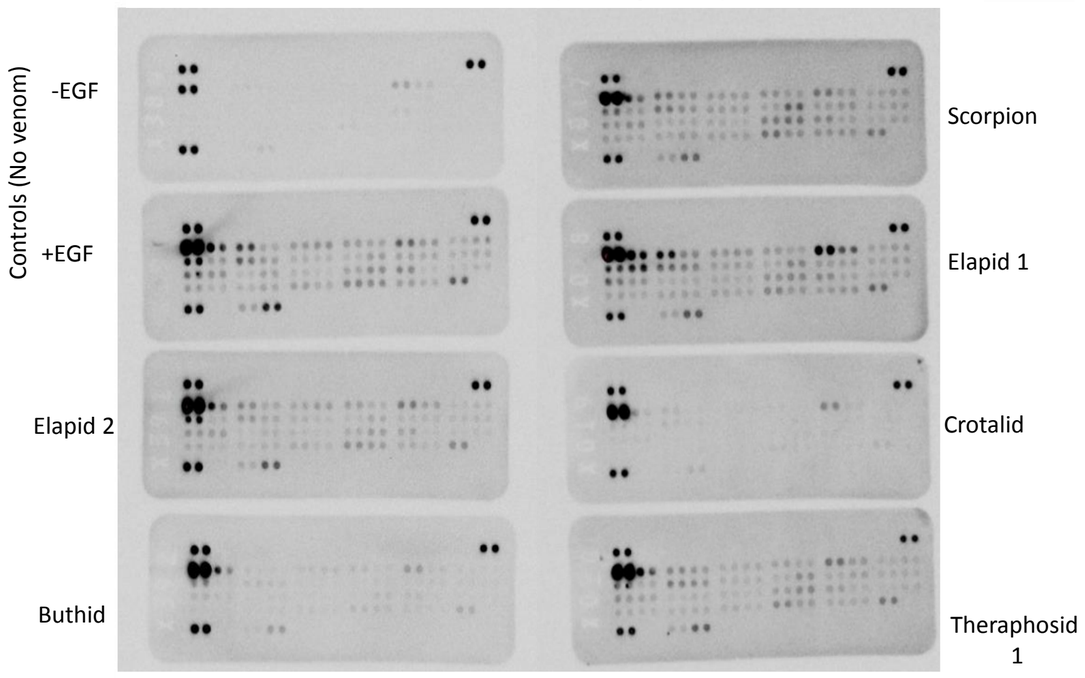
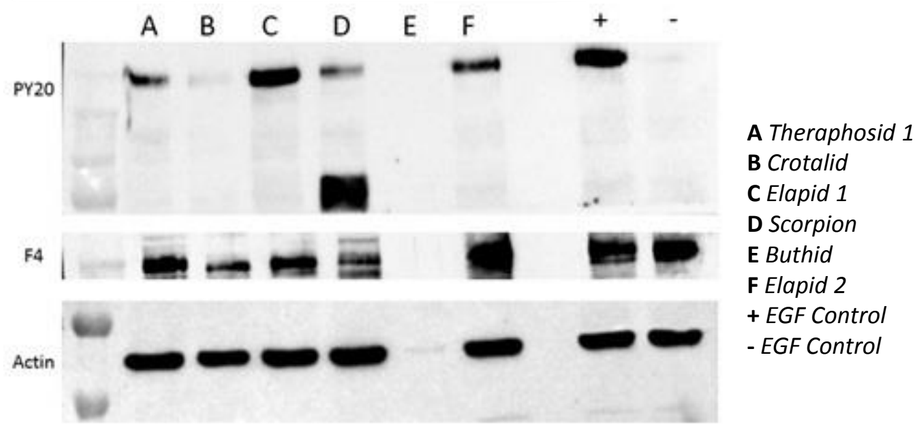


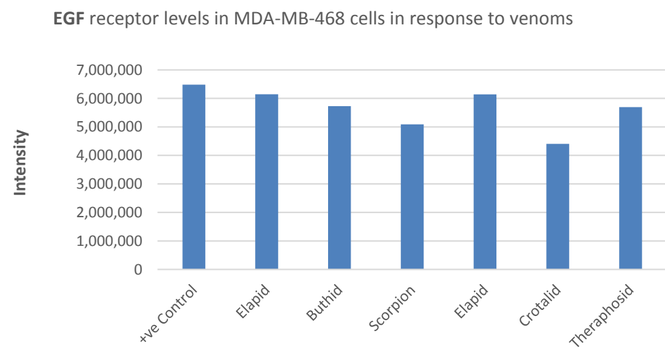
Figure 3B: Western Blots of Lysates used on Kinome Blots



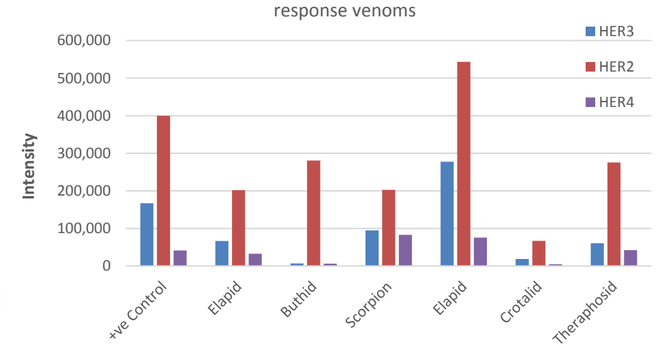
A *Theraphosid 1*
B *Crotalid*
C *Elapid 1*
D *Scorpion*
E *Buthid*
F *Elapid 2*
+ *EGF Control*
- *EGF Control*

Figure 4:- Kinome Arrays Analysis:-

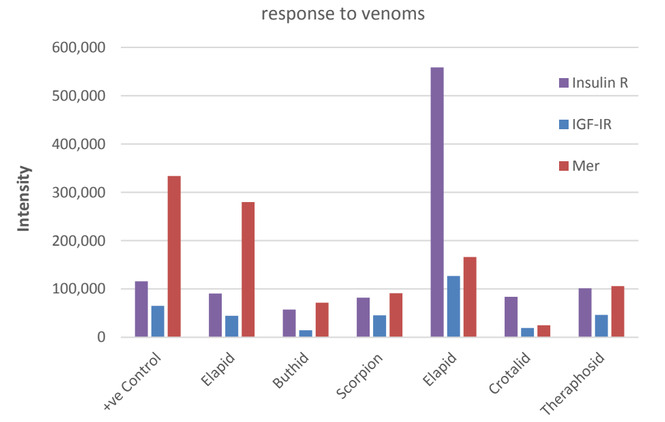
EGF receptor levels in MDA-MB-468 cells in response to venoms



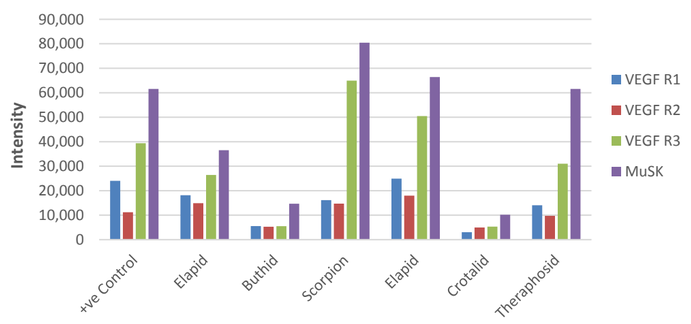
HER2, HER3 and HER4 receptor levels in MDA-MB-468 cells in response venoms



Insulin, **IGF-IR** and **Mer** receptor levels in MDA-MB-468 cells in response to venoms



VEGF R1, VEGFR 2, VEGF R3 and MuSK receptor levels in MDA-MB-468 cells in response venoms



Discussion

- Crude venom western blots show receptor activity inhibition at 1/50, 1/100 and 1/1000 treatment dilutions *(Fig. 1)*.
- Fractionated Theraphosid 2 venom shows a mixture of components that both upregulate (f6v2) and downregulate (f5, f15, f16) EGF Receptor phosphorylation levels *(Fig. 2)*
- When compared to the positive control, the Kinome blots show a range of changes (both up/down regulations) in many of the RTKs in response to different venoms. It is difficult to ascertain whether these are changes in phosphorylation levels or total receptor levels *(Fig. 3A)*
- In particular both EGFR phosphorylation and/or protein levels are decreasing in response to Theraphosid 1, Crotalid and Elapid 2 venoms. Potential EGFR degradation my be occurring in response to Scorpion venom *(Fig. 3B)*

Canterbury Christ Church University

VENOMTECH® Drug Discovery Solutions™

Kent cancer trust Supporting research for Kent