**The endogenous calpain inhibitor Calpastatin attenuates axon degeneration in murine Guillain-Barré syndrome.**

Study Aim: This study used established *ex* and *in vivo* acute and subacute mouse models of axonal Guillain-Barre syndrome (GBS)(AMAN) to study the therapeutic advantages of calpain inhibition to protect axon integrity and recovery.

For immunostaining, images were captured using Zeiss LSM or Zeiss AxioImager Z1 microscopes and observed using Zeiss Zen or ImageJ (FIJI) software. Quantification was performed using the same. Analysis output can be found in Excel sheets. Statistical analysis was performed using GraphPad software. The same experimental names are given for linked Excel and Graphpad files. Details of experimental names are provided below.

Figure 1. A-B.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Expt name | Mice | Tissue | Purpose | Staining and output |
| RM C34 | WT n=3,  *hCAST* n=3 | Ex vivo TS  (half 0 min, half 60 min) | Assess Ab binding under different conditions *ex vivo* | Bungarotoxin (BTx)  IgG3  Confocal .lsm files |

Figure 1 C-E.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Expt name | Mice | Tissue | Purpose | Staining and output |
| RM C11 | WT n=3,  *hCAST* n=3 | Ex vivo TS  (half injured, half control) | Assess axon integrity following acute injury with AgAb and complement *ex vivo* | Neurofilament (NF-H)  Myelin basic protein (MBP)  BTx  Membrane attack complex (MAC)  AxioImager .czi files |

Figure 2.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Expt name | Mice | Tissue | Purpose | Staining and output |
| RM C15  RM C32 | WT n=3,  *hCAST* n=3 | Ex vivo TS  (half injured, half control) | Assess nodal protein integrity following acute injury with AgAb and complement *ex vivo* | Nav1.6 or AnkyrinG  Myelin basic protein (MBP)  BTx  Membrane attack complex (MAC)  AxioImager .czi files |

Figure 3.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Expt name | Mice | Tissue | Purpose | Staining and output |
| RM C17 | WT n=4,  *hCAST* n=4  Naïve n=4 | Diaphragm for immun (B) and serum for SIMOA (C) | Assess respiratory function and axon integrity following acute injury with AgAb and complement *in vivo* | Respiratory output from eDaq software .csv files  NFH, BTx, MAC  Confocal .lsm files |

Figure 4.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Expt name | Mice | Tissue | Purpose | Staining and output |
| RM C28 | WT n=3,  *hCAST* n=4  Naïve n=4 | Diaphragm | Assess nodal protein integrity following acute injury with AgAb and complement *in vivo* | Nav1.6 or AnkyrinG  MBP, BTx, MAC  AxioImager .czi files |

Figure 5.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Expt name | Mice | Tissue | Purpose | Staining and output |
| RM C28 | WT n=4,  *hCAST* n=4  Naïve n=4 | serum for SIMOA  (Diaphragm collected ) | Assess respiratory function and axon integrity following sub-acute injury with AgAb and complement *in vivo* | Respiratory output from eDaq software .csv files  Simoa |

Codes :

**RM C15 repeat May 2019**

|  |  |
| --- | --- |
| **A** | WT Ab 1 |
| **B** | hCAST 2 AB |
| **C** | WT 1 INJ |
| **D** | hCAST 1 AB |
| **E** | hCAST 2 INJ |
| **F** | WT 2 INJ |
| **G** | hCAST 1 INJ |
| **H** | WT 2 AB |

**RM C32 ex vivo 4h MOG1 injury (AnkG)**

|  |  |
| --- | --- |
| A (m1) | hCAST |
| B (m2) | WT |
| C (m3) | hCAST |
| D (m4) | WT |
| E (m5) | WT |
| F (m6) | hCAST |

**RM C17 (DIA cut and stained by Hannah and Iliyana and reimaged by me on confocal)**

MOG1 hCAST injury

|  |  |
| --- | --- |
| A | **hCAST Ab 1** |
| B | **WT INJ 3** |
| C | **WT INJ 4** |
| D | **WT INJ 1** |
| E | **WT Ab 1** |
| F | **hCAST INJ 4** |
| G | **hCAST INJ 2** |
| H | **WT INJ 2** |
| I | **hCAST INJ 1** |
| J | **WT Ab 2** |
| K | **hCAST INJ 3** |
| L | **hCAST Ab 2** |

**RM C28 Aug 2021 acute hCAST MOG1**

|  |  |  |
| --- | --- | --- |
| **A** | **M5** | **hCAST 3** |
| B | M3 | **hCAST 2** |
| C | H (EXPT 1) | **CON 2** |
| D | M | **CON 3** |
| E | R | **CON 4** |
| F | D | **CON 1** |
| G | M4 | **WT 2** |
| H | M1 | **WT 1** |
| I | M6 | **hCAST 4** |
| J | M2 | **hCAST 1** |
| K | M7 | **WT 3** |

**RM C34 April 2021 iinternalisation ex vivo MOG1 WT vs hCAST**

|  |  |
| --- | --- |
| A | hCAST 0MIN |
| B | WT 0" 1 |
| C | HCAST 60" 1 |
| D | HCAST 0" 2 |
| E | WT 60" 1 |
| F | WT 60" 2 |
| G | WT 0" 2 |
| H | Hcast 60" 2 |
| I | Hcast 0" 3 |
| J | Hcast 60" 3 |
| K | Wt 60" 3 |
| L | WT 0" 3 |

SIMOA codes

What I sent to Michael Chou from first round RM C17:

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **TUBE LABEL** | **EXPT #** | **Tissue treatment** |
| Sample 1 | M7 BLUE DOT | RM C16 | Naïve 1 |
| Sample 2 | M8 BLUE DOT | RM C16 | WT injury 1 |
| Sample 3 | M1 BLUE DOT | RM C17 March | hCAST injury 1 |
| Sample 4 | M5 BLUE DOT | RM C17 March | hCAST injury 2 |
| Sample 5 | M2 BLUE DOT |  | WT injury 2 |
| Sample 6 | M3 BLUE DOT |  | WT injury 3 |
| Sample 7 | M4 BLUE DOT |  | WT injury 4 |
| Sample 8 | M1 (115) BLUE DOT | RM C17 (3) June | hCAST injury 3 |
| Sample 9 | M2 (116) BLUE DOT | RM C17 (3) June | hCAST injury 4 |
| Sample 10 | M6.2 RED SPOT | RM C17 | Naïve control |
| Sample 11 | M7.2 RED SPOT | RM C17 | Naïve control |
| Sample 12 | M8.2 RED SPOT | RM C17 | hCAST control |
| Sample 13 | M9.2 RED SPOT | RM C17 | hCAST control |

What I sent to Duncan from Second round RM C17 (RM C28 acute):

|  |  |  |
| --- | --- | --- |
| **#** | **TUBE LABEL** | **Tissue treatment** |
| Sample 1 | control 1 yellow sticker | RMC27/28 hCAST |
| Sample 2 | control 2 yellow sticker | RMC27/28 hCAST |
| Sample 3 | control 3 yellow sticker | RMC27/28 hCAST |
| Sample 4 | control 4 yellow sticker | RMC27/28 hCAST |
| Sample 5 | M1 | RM C28 hCAST |
| Sample 6 | M2 | RM C28 hCAST |
| Sample 7 | M3 | RM C28 hCAST |
| Sample 8 | M4 | RM C28 hCAST |
| Sample 9 | M5 | RM C28 hCAST |
| Sample 10 | M6 | RM C28 hCAST |
| Sample 11 | M7 | RM C28 hCAST |

What I will send to Michael from RM C28 sub-acute:

|  |  |  |
| --- | --- | --- |
| **#** | **TUBE LABEL** | **Tissue treatment** |
| Sample 1 | June M3 YELLOW SPOT | Thy1 INJ 1 |
| Sample 2 | June M4 YELOW SPOT | hCAST INJ 1 |
| Sample 3 | June M5 YELOW SPOT | Naïve 1 |
| Sample 4 | July M2 PINK SPOT | hCAST INJ 2 |
| Sample 5 | July M3 PINK SPOT | hCAST INJ 3 |
| Sample 6 | July M5 PINK SPOT | hCAST INJ 4 |
| Sample 7 | July M6 PINK SPOT | Naïve 2 |
| Sample 8 | July M7 PINK SPOT | Naïve 3 |
| Sample 9 | July M8 PINK SPOT | Thy1 INJ 2 |
| Sample 10 | August M1 BLUE SPOT | Naïve 4 |
| Sample 11 | August M2 BLUE SPOT | Naïve 5 |
| Sample 12 | August M3 BLUE SPOT | Thy1 INJ 3 |
| Sample 13 | August M5 BLUE SPOT | Thy1 INJ 4 |